

LAMPIRAN

Lampiran 1 Uji Statistik

Descriptive Statistics

	Mean	Std. Deviation	N
Segera	60,117	,2137	6
1 hari	61,783	,1169	6
2 hari	64,700	,1673	6
3 hari	65,517	,1169	6

Tests of Normality

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Standardized Residual for Segera	,207	6	,200*	,890	6	,317
Standardized Residual for Disimpan_1_hari	,223	6	,200*	,908	6	,421
Standardized Residual for Disimpan_2_hari	,333	6	,036	,873	6	,238
Standardized Residual for Disimpan_3_hari	,223	6	,200*	,908	6	,421

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Mauchly's Test of Sphericity^a

Measure: Kadar_Kolesterol_HDL

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon ^b		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Waktu	,368	3,723	5	,601	,590	,891	,333

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.

a. Design: Intercept

Within Subjects Design: Waktu

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Measure: Kadar_Kolesterol_HDL

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Waktu	Sphericity Assumed	114,085	3	38,028	1739,536	,000
	Greenhouse-Geisser	114,085	1,771	64,414	1739,536	,000
	Huynh-Feldt	114,085	2,672	42,701	1739,536	,000
	Lower-bound	114,085	1,000	114,085	1739,536	,000
Error(Waktu)	Sphericity Assumed	,328	15	,022		
	Greenhouse-Geisser	,328	8,856	,037		
	Huynh-Feldt	,328	13,359	,025		
	Lower-bound	,328	5,000	,066		

Pairwise Comparisons

Measure: Kadar_Kolesterol_HDL

(I) Waktu	(J) Waktu	Mean Difference (I-J)	95% Confidence Interval for Difference ^b			
			Std. Error	Sig. ^b	Lower Bound	Upper Bound
1	2	-1,667*	,117	,000	-2,162	-1,171
	3	-4,583*	,087	,000	-4,951	-4,215
	4	-5,400*	,106	,000	-5,849	-4,951
2	1	1,667*	,117	,000	1,171	2,162
	3	-2,917*	,065	,000	-3,193	-2,641
	4	-3,733*	,056	,000	-3,969	-3,498
3	1	4,583*	,087	,000	4,215	4,951
	2	2,917*	,065	,000	2,641	3,193
	4	-817*	,060	,000	-1,070	-563
4	1	5,400*	,106	,000	4,951	5,849
	2	3,733*	,056	,000	3,498	3,969
	3	,817*	,060	,000	,563	1,070

Based on estimated marginal means

*. The mean difference is significant at the ,05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Lampiran 2 Monitoring Suhu Ruang

Tanggal	Waktu		Suhu (°C)
Selasa 22 Juni 2021	Pagi	08:00	20,1
	Sore	16:26	23,1
Rabu 23 Juni 2021	Pagi	08:00	22,0
	Sore	16:35	24,1
Kamis 24 Juni 2021	Pagi	08:00	21,1
	Sore	16:42	23,6
Jum'at 25 Juni 2021	Pagi	08:00	22,0
	Sore	16:30	23,9

Lampiran 3 Dokumentasi Penelitian

A. Alat dan Bahan Penelitian



Gambar 1. Cobas C311



Gambar 2. Tip Biru



Gambar 3. Mikropipet



Gambar 4. Tabung Vakum



Gambar 5. Jarum Vakum



Gambar 6. Alkohol Swab



Gambar 6. Holder



Gambar 7. Tourniquet



Gambar 8. Sampel darah



Gambar 9. Termometer

Gambar 10. *Sample cup*

Gambar 11. Reagen Kolesterol HDL



Gambar 12. Bahan Kontrol



Gambar 13. *Sentrifuge*



Gambar 14. Komputer

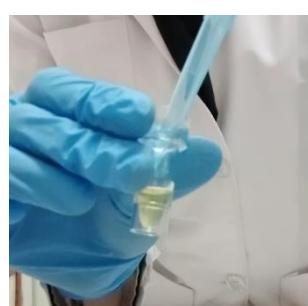
B. Prosedur Penelitian



Gambar 15. Monitoring suhu



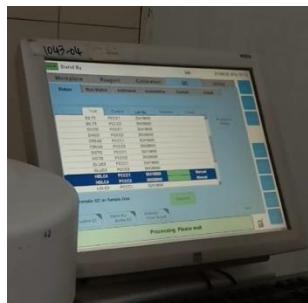
Gambar 16. Sentrifuge Sampel



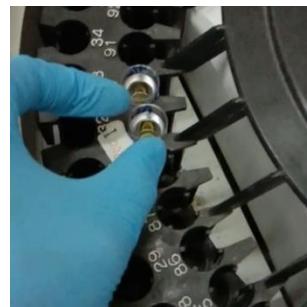
Gambar 17. Pemisahan serum



Gambar 18. Distribusi serum untuk pengulangan



Gambar 19. Pemilihan Pemeriksaan Kontrol Serum



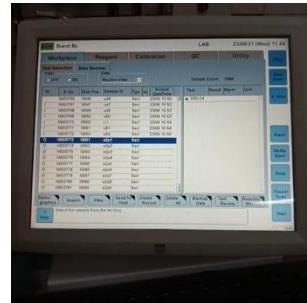
Gambar 20. Memasukkan kontrol Ke Tray Cobas C311



Gambar 21. Order Sampel



Gambar 22. Simpan Sampel pada Tray Cobas C311



Gambar 23. Hasil Pemeriksaan

Lampiran 4 Informed Consent***Informed Consent*****KEMENTERIAN KESEHATAN REPUBLIK INDONESIA****POLITEKNIK KESEHATAN KEMENKES BANDUNG****JURUSAN TEKNOLOGI LABORATORIUM MEDIS**

LEMBAR *INFORMED CONSENT*

Penelitian ini berjudul “Pengaruh Waktu Penyimpanan Serum Terhadap Kadar Kolesterol *High Density Lipoprotein* Pada Suhu Ruang” bermaksud untuk mengetahui pengaruh waktu penyimpanan serum terhadap kadar kolesterol baik yang ada didalam tubuh. Penelitian ini bermanfaat untuk memberikan informasi terhadap instansi dan masyarakat tentang pengaruh waktu penyimpanan serum terhadap kadar kolesterol baik pada suhu ruang. Sehingga sebagai bahan pertimbangan dalam penyimpanan sampel pemeriksaan kolesterol HDL atau kolesterol baik guna membantu dalam menegakkan diagnosis kimia klinik yang akurat.

Untuk melakukan penelitian ini, saya memerlukan bantuan saudara/i yang merupakan sampel penelitian saya. Karenanya saya meminta bantuan saudara/i agar berkenan untuk dilakukan pengambilan darah. Selanjutnya akan dilakukan pengambilan darah sebanyak 15 mL yang kemudian darah tersebut akan diperiksa kadar kolesterol baik didalam darah. Dalam proses pengambilan darah, akan ada sedikit rasa nyeri dan resiko kecil terjadinya pembiruan di kulit sekitar bekas tusukan jarum. Jika terjadi sesuatu yang memerlukan pertolongan dokter pada saat

pengambilan darah, maka saudara/i akan segera diberi pertolongan. Bila perlu dirujuk ke rumah sakit dan biaya akan ditanggung oleh peneliti.

Hasil pemeriksaan saudara akan dijaga kerahasiaannya. Jika diperlukan untuk kepentingan kedinasan, identitas saudara akan dilindungi dalam bentuk kode atau nomer dan tidak akan diketahui oleh siapapun dalam pengambilan data tersebut.

Partisipasi saudara/i ini bersifat sukarela, semua biaya penelitian ini dibebankan kepada peneliti. Adapun segala informasi atau catatan mengenai hasil pemeriksaan saudara/i akan dijaga kerahasiaannya. Kalaupun diperlukan untuk kepentingan kedinasan, maka nama anda akan dilindungi dalam bentuk kode atau nomor yang tidak akan diketahui oleh siapapun baik yang turut dan tidak turut dalam pengambilan data tersebut.

Pertanyaan

Apabila ada pertanyaan yang menyangkut penelitian dan diri Saudara/i, dapat hubungi langsung kepada Ilma Nur'afni Soderi, Jurusan Teknologi Laboratorium Medis Politeknik Kesehatan Kemenkes Bandung, Jalan Babakan Loa Cimahi.

Telp. 081909822697

Lampiran 5 Persetujuan Setelah Penjelasan**LEMBAR PERSETUJUAN SETELAH PENJELASAN**

Saya telah diberikan penjelasan dan telah diberi kesempatan bertanya atas apa yang tidak saya mengerti. Saya mengerti, bahwa partisipasi saya dilakukan secara sukarela, dan dapat menolak atau mengundurkan diri sewaktu – waktu tanpa sanksi apapun dalam penelitian “Pengaruh Waktu Penyimpanan Serum Terhadap Kadar Kolesterol *High Density Lipoprotein Pada Suhu Ruang*” yang dilakukan oleh Ilma Nur’afni Soderi dari Jurusan Teknologi Laboratorium Medis Politeknik Kesehatan Kemenkes Bandung.

Saya memahami maksud, manfaat, resiko, waktu, dan prosedur penelitian ini, serta saya setuju dengan kompensasi yang akan saya terima. Saya akan membubuhkan tanda tangan saya di bawah ini dan menyatakan keikutsertaan saya dalam pelaksanaan penelitian ini.

Setelah membaca pernyataan di atas, saya yang bertanda tangan di bawah ini:

Nama :

Jenis Kelamin :

Umur :

Alamat :

No. Hp :

Telah bersedia untuk ikut serta menjadi subjek penelitian ini. Saya yakin yang saya sampaikan ini terjamin kebenarannya.

Bandung, 2021

Peneliti,

Responden,

Ilma Nur’afni Soderi
NIM. P17334117439

.....

Lampiran 6 Data Persetujuan Responden

LEMBAR PERSETUJUAN SETELAH PENJELASAN

Saya telah diberikan penjelasan dan telah diberi kesempatan bertanya atas apa yang tidak saya mengerti. Saya mengerti, bahwa partisipasi saya dilakukan secara sukarela, dan dapat menolak atau mengundurkan diri sewaktu-waktu tanpa sanksi apapun dalam penelitian "Pengaruh Waktu Penyimpanan Serum Terhadap Kadar Kolesterol High Density Lipoprotein Pada Suhu Ruang" yang dilakukan oleh Ilma Nur'afni Soderi dari Jurusan Analis Kesehatan Poltekkes Kemenkes Bandung.

Saya memahami maksud, manfaat, resiko, waktu, dan prosedur penelitian ini, serta saya setuju dengan kompensasi yang akan saya terima. Saya akan membubuhkan tanda tangan saya di bawah ini dan menyatakan keikutsertaan saya dalam pelaksanaan penelitian ini.

Setelah membaca pernyataan di atas, saya yang bertanda tangan di bawah ini:

Nama : Syifa Aulia Maaghfira

Jenis Kelamin : Perempuan

Umur : 21 tahun

Alamat : Jl. Kalijati 6 no. 36a, Antapani Kulon, Bandung

No. Hp : 089 507 820 534

Telah bersedia untuk ikut serta menjadi subjek penelitian ini. Saya yakin yang saya sampaikan ini terjamin kebenarannya.

Bandung, 2021

Peneliti,

Ilma Nur'afni Soderi
NIM. P17334117439

Responden,

Syifa Aulia Maaghfira

Lampiran 7 Etik Penelitian

	 <p>KOMISI ETIK PENELITIAN KESEHATAN HEALTH RESEARCH ETHICS COMMITTEE</p> <p>POLITEKNIK KESEHATAN KEMENTERIAN KESEHATAN BANDUNG</p>	
KETERANGAN LAYAK ETIK DESCRIPTION OF ETHICAL APPROVAL "ETHICAL APPROVAL"		
No. 26/KEPK/EC/VII/2021		
<p>Protokol penelitian yang diusulkan oleh <i>The research protocol proposed by</i></p> <p>Peneliti utama : Ilma Nur'afni Soderi <i>Principal Investigator</i></p> <p>Nama Institusi : Jurusan Teknologi Laboratorium Medik <i>Name of the Institution</i> Poltekkes Kemenkes Bandung</p> <p>Dengan judul: <i>Title</i></p> <p>"PENGARUH WAKTU PENYIMPANAN SERUM TERHADAP KADAR KOLESTEROL HIGH DENSITY LIPOPROTEIN PADA SUHU RUANG"</p> <p>"EFFECT OF SERUM STORAGE TIME ON HIGH DENSITY LIPOPROTEIN CHOLESTEROL AT ROOM TEMPERATURE"</p> <p>Dinyatakan layak etik sesuai 7 (tujuh) Standar WHO 2011, yaitu 1) Nilai Sosial, 2) Nilai Ilmiah, 3) Pemerataan Beban dan, 4) Risiko, 5) Bujukan/Eksplorasi, 6) Kerahasiaan dan Privacy, dan 7) Persetujuan Setelah Penjelasan, yang merujuk pada Pedoman CIOMS 2016. Hal ini seperti yang ditunjukkan oleh terpenuhinya indikator setiap standar.</p> <p><i>Declared to be ethically appropriate in accordance to 7 (seven) WHO 2011 Standards, 1) Social Values, 2) Scientific Values, 3) Equitable Assessment and Benefits, 4) Risks, 5) Persuasion/Exploitation, 6) Confidentiality and Privacy, and 7) Informed Consent, referring to the 2016 CIOMS Guidelines. This is as indicated by the fulfillment of the indicators of each standard.</i></p> <p>Pernyataan Laik Etik ini berlaku selama kurun waktu tanggal 26 Juli 2021 sampai dengan tanggal 26 Juli 2022. <i>This declaration of ethics applies during the period July 26, 2021 until July 26, 2022.</i></p>		
 <p>July 26, 2021 <i>Professor and Chairperson,</i> Dr. Suparmi, SKM., M.Sc.</p>		

Lampiran 8 Kit Insert

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HDLC4

HDL-Cholesterol Gen.4

Order information

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
07528566190	HDL-Cholesterol Gen.4 (350 tests)	System-ID 07 7589 4
12172623 122	Calibrator l.a.s. Lipids (3 x 1 mL)	Code 424
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392
04489357 190	Diluent NaCl 9% (50 mL)	System ID 07 6869 3

English

System information

For cobas c 311/501 analyzers:

HDLC4: ACN 454

For cobas c 502 analyzer:

HDLC4: ACN 8454

Intended use

In vitro diagnostic test for the quantitative determination of the HDL-cholesterol concentration in human serum and plasma on Roche/Hitachi cobas c systems.

Summary

High density lipoproteins (HDL) are responsible for the reverse transport of cholesterol from the peripheral cells to the liver. In the liver, cholesterol is transformed to bile acids which are then excreted into the intestine via the biliary tract.

Monitoring of HDL-cholesterol in serum or plasma is of clinical relevance as the HDL-cholesterol concentration is important in the assessment of atherosclerotic risk. Elevated HDL-cholesterol concentrations protect against coronary heart disease (CHD), whereas reduced HDL-cholesterol concentrations, particularly in conjunction with elevated triglycerides, increase cardiovascular risk.¹

Two cholesterol related variables that are predictive of cardiovascular disease (CVD) have emerged. These are non-HDL-cholesterol^{2,3,4} (= cholesterol - HDL-cholesterol) and the rate of cholesterol transfer from the macrophages to HDL, also described as cholesterol efflux capacity.⁵ Whereas both cholesterol and HDL-cholesterol can be readily determined with high accuracy, currently, non-HDL-cholesterol appears to be best suited for patient management.

A variety of methods are available to determine HDL-cholesterol, including ultracentrifugation (reference method in combination with cholesterol measurement by the Abell-Kendall method), electrophoresis, HPLC, precipitation, and direct methods.⁶ Of these, the direct methods are used routinely. Roche HDLC4 is also a direct method. The automated HDLC4 assay uses detergents, cholesterol esterase (CHER), cholesterol oxidase (CHOD) and peroxidase to form a colored pigment that is measured optically.^{7,8}

The HDLC4 assay meets the 1998 National Institutes of Health (NIH) / National Cholesterol Education Program (NCEP) goals for precision and accuracy.^{9,10}

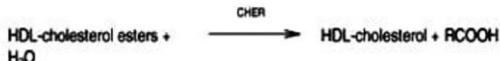
Test principle^{7,8}

Homogeneous enzymatic colorimetric test.

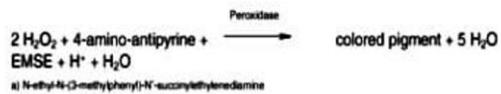
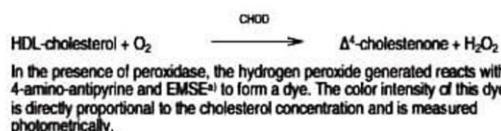
Non-HDL lipoproteins such as LDL, VLDL and chylomicrons are combined with polyanions and a detergent forming a water-soluble complex. In this complex the enzymatic reaction of CHER and CHOD towards non-HDL lipoproteins is blocked.

Finally only HDL-particles can react with CHER and CHOD. The concentration of HDL-cholesterol is determined enzymatically by CHER and CHOD.

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by CHER.



In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4 -cholesteneone and hydrogen peroxide.



Reagents - working solutions

R1 TAPSO^{b)} buffer: 62.1 mmol/L, pH 7.77; polyanion: 1.25 g/L; EMSE: 1.08 mmol/L; ascorbate oxidase (cucurbita): $\geq 50 \mu\text{kat/L}$; peroxidase (horseradish): $\geq 166.7 \mu\text{kat/L}$; detergent; BSA: 2.0 g/L; preservative

R2 Bis-Tris^{c)} buffer: 20.1 mmol/L, pH 6.70; cholesterol esterase (microorganism): $\geq 7.5 \mu\text{kat/L}$; cholesterol oxidase (recombinant E. coli): $\geq 7.17 \mu\text{kat/L}$; cholesterol oxidase (microorganism): $\geq 76.7 \mu\text{kat/L}$; peroxidase (horseradish): $\geq 333 \mu\text{kat/L}$; 4-amino-antipyrine: 1.48 mmol/L; BSA: 3.0 g/L; detergents; preservative

b) 2-Hydroxy-N-(3-hydroxymethyl)imethyl-3-aminopropanesulfonic acid

c) Bis(2-hydroxyethyl)iminotriis(hydroxymethyl)methane

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

Reagent handling

Ready for use

The intrinsic color of the reagent does not interfere with the test.

Storage and stability

HDLC4

Shell life at 2-8 °C: See expiration date on cobas c pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

Diluent NaCl 9%

Shell life at 2-8 °C: See expiration date on cobas c pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

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HDLC4

HDL-Cholesterol Gen.4

cobas®

Only the specimens listed below were tested and found acceptable.
Serum.

Plasma: Li-heparin, K₂- and K₃-EDTA plasma.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Collect blood by using an evacuated tube or syringe. Specimens should preferably be analyzed on the day of collection.

Fasting and non-fasting samples can be used.^{11,12}

Stability in serum:
72 hours at 15-25 °C¹³
7 days at 2-8 °C¹³

12 months at -20 °C¹⁴

24 months at -70 °C¹⁵

Stability in Li-heparin, K₂- and K₃-EDTA plasma:
72 hours at 15-25 °C¹³
7 days at 2-8 °C¹³

3 months at (-15)-(-25) °C¹³

18 months at -70 °C¹³

18 months at -80 °C¹⁶

It is reported that EDTA stabilizes lipoproteins.¹⁷

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma**cobas c 311 test definition**

Assay type 2-Point End

Reaction time / Assay points 10/6-33

Wavelength (sub/main) 700/600 nm

Reaction direction Increase

Units mmol/L (mg/dL)

Reagent pipetting Diluent (H₂O)

R1 120 µL –

R2 40 µL –

Sample volumes **Sample** **Sample dilution**

Sample **Diluent (NaCl)**

Normal 2.4 µL – –

Decreased 12.5 µL 15 µL 135 µL

Increased 2.4 µL – –

cobas c 501 test definition

Assay type 2-Point End

Reaction time / Assay points 10/10-47

Wavelength (sub/main)	700/600 nm	
Reaction direction	Increase	
Units	mmol/L (mg/dL)	
Reagent pipetting	Diluent (H ₂ O)	
R1	120 µL	–
R2	40 µL	–

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)

Normal	2.4 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	2.4 µL	–	–

cobas c 502 test definition

Assay type 2-Point End

Reaction time / Assay points 10/10-47

Wavelength (sub/main) 700/600 nm

Reaction direction Increase

Units mmol/L (mg/dL)

Reagent pipetting Diluent (H₂O)

R1 120 µL –

R2 40 µL –

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)

Normal	2.4 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	2.4 µL	–	–

Calibration

Calibrators S1: H₂O

S2: C.I.a.s. Lipids

Calibration mode Linear

Calibration frequency 2-point calibration

* after reagent lot change

* as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the designated CDC reference method (ultracentrifugation method).⁹ The standardization meets the requirements of the "HDL Cholesterol Method Evaluation Protocol for Manufacturers" of the US National Reference System for Cholesterol, CRMLN (Cholesterol Reference Method Laboratory Network), November 1994.¹³

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Quality control materials are intended for use only as monitors of accuracy and precision.

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HDLC4

HDL-Cholesterol Gen.4

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factors:

$$\text{mmol/L} \times 38.66 = \text{mg/dL}$$

$$\text{mg/dL} \times 0.0259 = \text{mmol/L}$$

Limitations - interference¹⁸

Criterion: Recovery within $\pm 10\%$ of initial value at a HDL-cholesterol concentration of 1 mmol/L (38.7 mg/dL).

Icterus:¹⁹ No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 $\mu\text{mol/L}$ or 60 mg/dL).

Hemolysis:¹⁹ No significant interference up to an H index of 1200 (approximate hemoglobin concentration: 745 $\mu\text{mol/L}$ or 1200 mg/dL).

Lipemia (Intralipid):¹⁹ No significant interference up to an L index of 2000. No significant interference from native triglycerides up to 13.7 mmol/L or 1200 mg/dL. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Other: Elevated concentrations of free fatty acids and denatured proteins may cause falsely elevated HDL-cholesterol results.

Ascorbic acid up to 2.84 mmol/L (50 mg/dL) does not interfere.

Abnormal liver function affects lipid metabolism; consequently, HDL and LDL results are of limited diagnostic value. In some patients with abnormal liver function, the HDL-cholesterol result may significantly differ from the DCM (designated comparison method) result due to the presence of lipoproteins with abnormal lipid distribution.²⁰

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{21,22}

Statins (Simvastatin) and fibrates (Benzaibibrate) tested at therapeutic concentration ranges did not interfere.

N-acetylcysteine: No significant interference up to a N-acetylcysteine concentration of 2.76 mmol/L (450 mg/L).

Acetaminophen intoxications are frequently treated with N-acetylcysteine. N-acetylcysteine at the therapeutic concentration when used as an antidote and the acetaminophen metabolite N-acetyl-p-benzozquinone imine (NAPO) independently may cause falsely low HDL-cholesterol results.

Metamizole: Venupuncture should be performed prior to the administration of metamizole. Venupuncture immediately after or during the administration of metamizole may lead to falsely low results.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.²³

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi cobas c systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. cobas c 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the cobas link, manual input is not required.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.08-3.88 mmol/L (3.09-150 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.08 mmol/L (3.09 mg/dL)

Limit of Detection = 0.08 mmol/L (3.09 mg/dL)

Limit of Quantitation = 0.08 mmol/L (3.09 mg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from $n \geq 60$ measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a precision of $\leq 30\% \text{ CV}$. It has been determined using low concentration HDL-cholesterol samples.

Expected values

	No risk	Moderate risk	High risk
Females ^{24,25,26}	> 1.68 mmol/L (> 65 mg/dL)	1.15-1.68 mmol/L (45-65 mg/dL)	< 1.15 mmol/L (< 45 mg/dL)
Males ^{24,25,26}	> 1.45 mmol/L (> 55 mg/dL)	0.90-1.45 mmol/L (35-55 mg/dL)	< 0.90 mmol/L (< 35 mg/dL)

National Cholesterol Education Program (NCEP) guidelines:²⁷

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

≥ 60 mg/dL: High HDL-cholesterol ("negative" risk factor for CHD)

HDL-cholesterol is affected by a number of factors, e.g. smoking, exercise, hormones, sex and age.

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

National Cholesterol Education Program (NCEP) guidelines are based on serum values. When classifying patients, serum or serum equivalent values should be used. Therefore the NCEP recommends using a factor of 1.03 to convert EDTA plasma values to serum values. A later study found EDTA plasma concentrations to be 4.7 % lower than those in serum.²⁸ To comply with the 1998 NCEP goal of a bias $< 5\%$ it is recommended that each laboratory validates the conversion factor and enters it into the test parameters for HDL-cholesterol.²⁹

Treatment goals for non-HDL-cholesterol have been proposed:²

	NCEP ATP III	ADA/AHA Guidelines for patients with increased cardiometabolic risk
Optional goal for very-high/highest risk patients (known CVD, diabetes with elevated risk)	< 3.37 mmol/L (< 130 mg/dL)	< 2.59 mmol/L (< 100 mg/dL)
Optional goal for those with established cardiovascular disease and multiple major risk factors	< 2.59 mmol/L (< 100 mg/dL)	
Optional goal for high-risk patients, CHD-risk-equivalent (Framingham 10-year risk score > 20 %/10 years, diabetes without other major risk factors)	< 3.37 mmol/L (< 130 mg/dL)	< 3.37 mmol/L (< 130 mg/dL)

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HDLC4

HDL-Cholesterol Gen.4

Optional goal for moderately-high/intermediate risk patients (≥ 2 major CVD risk factors, Framingham 10-year risk score from 10-20 %) < 4.14 mmol/L (< 160 mg/dL) < 3.37 mmol/L (< 130 mg/dL)

$$y = 0.980x + 0.013 \text{ mmol/L}$$

$$r = 0.973$$

$$r = 0.998$$

The sample concentrations were between 0.08 and 3.83 mmol/L (3.09 and 148 mg/dL).

Optional goal for high-risk patients, CHD-risk-equivalent (Framingham 10-year risk score $> 20\% / 10$ years, diabetes without other major risk factors) < 3.37 mmol/L (< 130 mg/dL)

HDL-cholesterol values for human serum samples obtained on a Roche/Hitachi cobas c 501 analyzer (y) were compared with those determined using the HDL Ultra Cholesterol Reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 111

Passing/Bablok³⁰ Linear regression

$$y = 0.957x - 0.024 \text{ mmol/L}$$

$$r = 0.944$$

$$r = 0.995$$

The sample concentrations were between 0.13 and 3.86 mmol/L (5.03 and 149 mg/dL).

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (4 aliquots per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	Mean	SD	CV
	mmol/L (mg/dL)	mmol/L (mg/dL)	%
PCCC Multi 1	0.73 (28.2)	0.004 (0.15)	0.6
PCCC Multi 2	1.76 (68.0)	0.01 (0.39)	0.6
Human serum 1	0.25 (9.67)	0.004 (0.15)	1.8
Human serum 2	1.05 (40.6)	0.01 (0.39)	0.7
Human serum 3	1.53 (59.1)	0.01 (0.39)	0.5
Human serum 4	2.05 (79.3)	0.01 (0.39)	0.6
Human serum 5	3.66 (141)	0.02 (0.77)	0.6
Intermediate precision	Mean	SD	CV
	mmol/L (mg/dL)	mmol/L (mg/dL)	%
PCCC Multi 1	0.73 (28.2)	0.01 (0.27)	1.0
PCCC Multi 2	1.72 (66.5)	0.02 (0.77)	1.4
Human serum 1	0.25 (9.67)	0.01 (0.19)	2.2
Human serum 2	1.05 (40.6)	0.01 (0.39)	0.8
Human serum 3	1.53 (59.1)	0.01 (0.39)	0.7
Human serum 4	2.05 (79.3)	0.02 (0.77)	0.8
Human serum 5	3.66 (141)	0.03 (1.16)	0.8

PCCC = PicciControl ClinChem

Method comparison

HDL-cholesterol values for human serum and plasma samples obtained on a Roche/Hitachi cobas c 701 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi cobas c 501 analyzer (x).

Sample size (n) = 59

Passing/Bablok ³⁰	Linear regression
$y = 1.006x + 0.032$ mmol/L	$y = 1.012x + 0.021$ mmol/L
$r = 0.994$	$r = 1.000$

The sample concentrations were between 0.11 and 3.69 mmol/L (4.25 and 143 mg/dL).

HDL-cholesterol values for human serum and plasma samples obtained on a COBAS INTEGRA 400 plus analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi cobas c 501 analyzer (x).

Sample size (n) = 118

Passing/Bablok ³⁰	Linear regression
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