LDHI2

cobas®

Lactate Dehydrogenase acc. to IFCC ver.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03004732 122	Lactate Dehydrogenase acc. to IFCC ver.2 300 tests	System-ID 07 6607 0	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171735 122	Precinorm U (4 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	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	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

LDHI2: ACN 080

LDIP2: ACN 147 (with automatic sample pre-dilution)[a)]

For **cobas c** 502 analyzer:

LDHI2: ACN 8080

LDIP2: ACN 8147 (with automatic sample pre-dilution)[a)]

a) Not available in the US

Intended use

In vitro test for the quantitative determination of lactate dehydrogenase in human serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5,6]

The lactate dehydrogenase (LDH) enzyme is widely distributed in tissue, particularly in the heart, liver, muscles and kidneys. The LDH in serum can be separated into five different isoenzymes based on their electrophoretic mobility. Each isoenzyme is a tetramer composed of two different subunits. These two subunits have been designated heart and muscle, based on their polypeptide chains. There are two homotetramers, LDH-1 (heart) and LDH-5 (muscle), and three hybrid isoenzymes.

Elevated serum levels of LDH have been observed in a variety of disease states. The highest levels are seen in patients with megaloblastic anemia, disseminated carcinoma and shock. Moderate increases occur in muscular disorders, nephrotic syndrome and cirrhosis. Mild increases in LDH activity have been reported in cases of myocardial or pulmonary infarction, leukemia, hemolytic anemia and non-viral hepatitis.

The method described here is derived from the formulation recommended by the IFCC[5,6] and was optimized for performance and stability.

Test principle

UV assay

Lactate dehydrogenase catalyzes the conversion of L-lactate to pyruvate; NAD is reduced to NADH in the process.

$$\text{L-Lactate} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

The initial rate of the NADH formation is directly proportional to the catalytic LDH activity. It is determined by photometrically measuring the increase in absorbance.

Reagents - working solutions

R1 N-methylglucamine: 400 mmol/L, pH 9.4 (37 °C); lithium lactate: 62 mmol/L; stabilizers; preservatives

R2 NAD: 62 mmol/L; stabilizers; preservatives

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

Storage and stability

LDHI2, LDIP2

Shelf 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 %

Shelf 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.

Only the specimens listed below were tested and found acceptable.
Serum (free from hemolysis).
Plasma: Li-heparin plasma. Plasma must be free from hemolysis and cells.

Caution: Plasma from primary tubes handled according to the manufacturer's instructions can still contain cells, leading to implausibly high results. One option for these cases is an application with automatic sample pre-dilution (ACN 147/ACN 8147). Alternatively it is recommended to transfer the plasma from the primary tube to a secondary sample tube.

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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.

Separate the serum or plasma from the clot or cells promptly.

Centrifuge samples containing precipitates before performing the assay.

Stability:[7] 7 days at 15-25 °C

The sample may be stored for 4 days at 2-8 °C or 6 weeks at -20 °C. In connection with certain diseases (e.g. hepatopathy, diseases of skeletal muscle, malignant tumors), the LDH-4 and LDH-5 isoenzyme portions are increased and unstable in cooled and frozen samples; this may lead to an incorrect LDH value in samples collected from patients suffering from such diseases.

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	Rate A		
Reaction time / Assay points	10 / 20-33		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes LDHI2	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	2.8 µL	–	–
Sample volumes LDIP2	*Sample*	*Sample dilution*	
		Sample	Diluent (NaCl)
Normal	14 µL	20 µL	80 µL
Decreased	5.6 µL	20 µL	80 µL
Increased	14 µL	20 µL	80 µL

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 28-47		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes LDHI2	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	2.8 µL	–	–
Sample volumes LDIP2	*Sample*	*Sample dilution*	
		Sample	Diluent (NaCl)
Normal	14 µL	20 µL	80 µL
Decreased	5.6 µL	20 µL	80 µL
Increased	14 µL	20 µL	80 µL

cobas c 502 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 28-47		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes LDHI2	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	5.6 µL	–	–
Sample volumes LDIP2	*Sample*	*Sample dilution*	
		Sample	Diluent (NaCl)
Normal	14 µL	20 µL	80 µL
Decreased	5.6 µL	20 µL	80 µL
Increased	20 µL	20 µL	80 µL

Calibration

Calibrators	S1: H_2O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • as required following quality control procedures

Traceability: This method has been standardized against the original IFCC[6] formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

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.

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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 factor: U/L x 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a lactate dehydrogenase activity of 200 U/L (3.34 µkat/L).

Icterus:[8] No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[8] No significant interference up to an H index of 15 (approximate hemoglobin concentration: 9.6 µmol/L or 15 mg/dL).

Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[8] No significant interference up to an L index of 900. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9,10]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[11]

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/Multiclean/SCCS or 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

10-1000 U/L (0.17-16.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:2.5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.5.

Lower limits of measurement

Lower detection limit of the test

10 U/L (0.17 µkat/L)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Expected values

Acc. to IFCC measured at 37 °C:[12]

Females	135-214 U/L	(2.25-3.55 µkat/L)
Males	135-225 U/L	(2.25-3.75 µkat/L)
Children (2-15 y)	120-300 U/L	(2.00-5.00 µkat/L)
Newborns (4-20 d)	225-600 U/L	(3.75-10.0 µkat/L)

Consensus values:[13]

Males & Females	up to 250 U/L	(up to 4.2 µkat/L)

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

Roche has not evaluated reference ranges in a pediatric population.

Specific performance data

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

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained:

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Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	164 (2.74)	1 (0.02)	0.8
Precipath U	263 (4.39)	2 (0.03)	0.7
Human serum 1	122 (2.04)	2 (0.03)	1.3
Human serum 2	396 (6.61)	4 (0.07)	0.9
Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	159 (2.66)	2 (0.03)	1.0
Precipath U	260 (4.34)	2 (0.03)	0.9
Human serum 3	117 (1.95)	3 (0.05)	2.7
Human serum 4	323 (5.39)	4 (0.07)	1.1

LDIP2

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	166 (2.77)	1 (0.02)	0.6
Precipath U	268 (4.48)	1 (0.02)	0.4
Human serum 1	125 (2.09)	1 (0.02)	1.1
Human serum 2	402 (6.71)	3 (0.05)	0.7
Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	168 (2.81)	2 (0.03)	1.1
Precipath U	272 (4.54)	3 (0.05)	0.9
Human serum 3	124 (2.07)	3 (0.05)	2.7
Human serum 4	340 (5.68)	4 (0.07)	1.2

Method comparison

LDH values for human serum and plasma samples obtained on a Roche/Hitachi **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

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Sample size (n) = 86

Passing/Bablok[14]	Linear regression
y = 1.000x + 4.40 U/L	y = 0.988x + 7.72 U/L
τ = 0.982	r = 1.000

The sample activities were between 100 and 935 U/L (1.67 and 15.6 µkat/L).

LDIP2

Sample size (n) = 86

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Passing/Bablok[14]	Linear regression
y = 1.000x + 6.82 U/L	y = 0.983x + 11.0 U/L
τ = 0.975	r = 0.999

The sample activities were between 89.8 and 950 U/L (1.50 and 15.9 µkat/L).

References

1 Thomas L, ed. Labor und Diagnose, 4th ed. Marburg: Die Medizinische Verlagsgesellschaft 1992.

2 Moss DW, Henderson AR, Kachmar JF. Enzymes. In: Tietz NW, ed. Fundamentals of Clinical Chemistry, 3rd ed. Philadelphia, PA: WB Saunders 1987;346-421.

3 Zimmerman HJ, Henry JB In: Henry JB, ed. Clinical Diagnosis and Management by Laboratory Methods. 17th ed.Philadelphia, PA: WB Saunders 1984;251-282.

4 Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;384-387.

5 van der Heiden C, Bais R, Gerhardt W, et al. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 8. IFCC method for lactate dehydrogenase. Eur J Clin Chem Clin Biochem 1994;32:639-655.

6 Schumann G, Bonora R, Ceriotti F, et al. IFCC Primary Reference Procedures for the Measurement of Catalytic Activity Concentrations of Enzymes at 37 °C – Part 3. Reference Procedures for the Measurement of Catalytic Concentrations of Lactate Dehydrogenase. Clin Chem Lab Med 2002;40(6):643-648.

7 Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB/99.1 Rev. 2. Jan. 2002.

8 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

9 Breuer J. Report on the Symposium “Drug effects in Clinical Chemistry Methods”. Eur J Clin Chem Clin Biochem 1996;34:385-386.

10 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

11 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

12 Lorentz K, Röhle G. Einführung der neuen Standardmethoden 1994 zur Bestimmung der katalytischen Enzymkonzentration bei 37 °C. Klin Chem Mitt 1995;26:290-293.

13 Thomas L, Müller M, Schumann G, et al Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005; 29(5):301-308.

14 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

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