LDHI2

Lactate Dehydrogenase acc. IFCC ver.2

cobas®

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057958190	Lactate Dehydrogenase acc. to IFCC ver.2 (850 tests)	System-ID 2081 001	**cobas c** 303, **cobas c** 503
10759350360	Calibrator f.a.s. (12 × 3 mL)	Code 20401	
05947626160	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 20391	
05947774160	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

For use in the USA only

System information

LDHI2: ACN 20810

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

R3 NAD: 62 mmol/L; stabilizers; preservatives

R1 is in position B and R3 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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: 1-800-428-2336

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	26 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
Plasma: Li-heparin plasma. Plasma must be free from cells.

Caution: Plasma from primary tubes handled according to the manufacturer's instructions can still contain cells, leading to implausibly high results. Alternatively it is recommended to transfer the plasma from the primary tube to a secondary sample tube.

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.

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

See the limitations and interferences section for details about possible sample interferences.

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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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	79 µL	–	
R3	16 µL	–	
Sample volumes LDHI2	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.2 µL	–	–
Decreased	2.8 µL	25.0 µL	56 µL
Increased	2.2 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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 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. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks.

Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

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

Calculation

cobas c systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a lactate dehydrogenase activity of 200 U/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 **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator’s manual.

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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 10 U/L (0.17 µkat/L)
Limit of Detection	= 10 U/L (0.17 µkat/L)
Limit of Quantitation	= 10 U/L (0.17 µkat/L)

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 ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the activity 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 activity samples.

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

The Limit of Quantitation is the lowest analyte activity that can be reproducibly measured with a total error of 20 %. It has been determined using low activity lactate dehydrogenase samples.

Expected values

U/L

Acc. to IFCC measured at 37 °C:[12]

Females	135-214 U/L
Males	135-225 U/L
Children (2-15 y)	120-300 U/L
Newborns (4-20 d)	225-600 U/L

Consensus values:[13]

Males & Females	up to 250 U/L

µkat/L

Acc. to IFCC measured at 37 °C:[12]

Females	2.25-3.55 µkat/L
Males	2.25-3.75 µkat/L
Children (2-15 y)	2.00-5.00 µkat/L
Newborns (4-20 d)	3.75-10.0 µkat/L

Consensus values:[13]

Males & Females	up to 4.2 µkat/L

Roche has not evaluated reference ranges in a pediatric population.

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

Specific performance data

Representative performance data on the analyzers are given below. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

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Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	172	1.06	0.6
PCCC2[b)]	294	1.35	0.5
Human serum 1	22.4	0.646	2.9
Human serum 2	164	1.29	0.8
Human serum 3	265	1.56	0.6
Human serum 4	520	2.09	0.4
Human serum 5	943	3.31	0.4

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	166	1.43	0.9
PCCC2[b)]	287	2.20	0.8
Human serum 1	22.4	0.779	3.5
Human serum 2	164	2.38	1.4
Human serum 3	265	2.32	0.9
Human serum 4	520	4.30	0.8
Human serum 5	943	5.65	0.6

Method comparison

Lactate dehydrogenase values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

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Sample size (n) = 66

Passing/Bablok[14]	Linear regression
y = 0.999x – 2.72 U/L	y = 1.001x – 3.32 U/L
τ = 0.992	r = 1.000

The sample activities were between 19.8 and 973 U/L.

Lactate dehydrogenase values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

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Sample size (n) = 60

Passing/Bablok[14]	Linear regression
y = 1.007x – 0.451 U/L	y = 1.016x – 3.51 U/L
τ = 0.982	r = 1.000

The sample activities were between 60.1 and 960 U/L.

References

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Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number

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