HBDH2

cobas®

α-Hydroxybutyrate Dehydrogenase Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
06750036190	06750036500	α-Hydroxybutyrate Dehydrogenase Gen.2, 100 tests	System-ID 07 6790 5	**cobas c** 311, **cobas c** 501/502 COBAS INTEGRA 400 plus

Materials required (but not provided):

		cobas c 311, **cobas c** 501/502	COBAS INTEGRA 400 plus
10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 401	System-ID 07 3718 6
12149435122	Precinorm U plus (10 x 3 mL)	Code 300	System-ID 07 7999 7
12149443122	Precipath U plus (10 x 3 mL)	Code 301	System-ID 07 8000 6
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	System-ID 07 7469 3
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	System-ID 07 7469 3
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	System-ID 07 7470 7
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	System-ID 07 7470 7

English

Intended use

In vitro test for the quantitative determination of α-hydroxybutyrate dehydrogenase (lactate dehydrogenase-1-isoenzyme) in serum and plasma on **cobas c** and COBAS INTEGRA systems.

Summary

α-hydroxybutyrate dehydrogenase (HBDH) activity measurements, performed with this assay in human serum and plasma are used as an aid for diagnosis of clinical conditions associated with cardiac tissue damage (e.g. myocardial infarction).

HBDH belongs to the lactate dehydrogenase (LDH) family. LDH is a key enzyme of anaerobic glucose metabolism catalyzing the reduction of pyruvate to lactate. It is a tetramer, where the 2 subunits (H, M) assemble to five different isoenzymes. According to their electrophoretic mobility, these isoenzymes are referred to as LDH1 (H4), LDH2 (H3M), LDH3 (H2M2), LDH4 (HM3), and LDH5 (M4). These isoenzymes have different substrate specificities, where LDH1 and LDH2 can also catalyze α-ketobutyrate (in place of pyruvate) to α-hydroxybutyrate at a higher rate than the other isoenzymes, which can be measured separately as hydroxybutyrate dehydrogenase activity, so that LDH1 and LDH2 are collectively also being termed HBDH.[1,2]

These enzymes are normally found intracellularly. Upon cell injury and/or necrosis, such as myocardial infarction, HBDH is released into the bloodstream, so that its elevation in blood is clinically used as an aid in diagnosing acute myocardial infarction. By using various substrates (e.g. α-ketobutyrate is used for HBDH), lactate dehydrogenases from the liver and the heart can be differentiated from each other.[3]

Several studies have shown that changes in the proportion of heart-specific LDH isoenzyme activities to the total LDH activity yield a reliable indication of the severity and progress of a recent myocardial infarction.[4,5] As the leakage of enzymes from cells into the serum starts early after infarction, HBDH can be used as an enzymatic marker of an acute myocardial infarction.[6]

Rotenberg et al. reported also that the measurement of heart-specific LDH isoenzymes 24 to 48 hours after heart surgery is a meaningful test for the diagnosis of perioperative myocardial infarction.[7,8]

Because of the even higher tissue specificity for the myocardium, the preferred biomarker for diagnosing myocardial injury is cardiac troponin, such as endorsed by the fourth universal definition of acute myocardial infarction.[9]

Test principle

UV test according to a standardized method.

α-hydroxybutyrate dehydrogenase catalyzes the conversion of α-ketobutyrate to α-hydroxybutyrate in a reaction where NADH is oxidized to NAD.

$$\alpha\text{-ketobutyrate} + NADH + H^+ \xrightarrow{\alpha\text{-}HBDH} \alpha\text{-hydroxybutyrate} + NAD^+$$

The rate of the NADH decrease is directly proportional to the α-HBDH activity and is measured photometrically.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

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, K_2-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.

Stability:[10] 3 days at 15-25 °C

7 days at 2-8 °C (activity decrease 5 %)

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.

Calculation

The systems automatically calculate the analyte concentration of each sample.

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

Expected values

72-182 U/L* (1.20-3.03 µkat/L**)[11]

HBDH2

α-Hydroxybutyrate Dehydrogenase Gen.2

*Calculated with a temperature conversion factor of 1.30 (25-37 °C).[12]

**calculated by unit conversion factor

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

cobas c systems

System information

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

HBDH2: ACN 567

For **cobas c** 502 analyzer:

HBDH2: ACN 8567

Reagents - working solutions

R1	Phosphate buffer: 68 mmol/L, pH 7.5 (25 °C); α-ketobutyrate: 3.7 mmol/L; preservative
R2	NADH: ≥ 1.1 mmol/L; preservative

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

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:	12 weeks

Application for serum and plasma

cobas c 311 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 17-24		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	2.8 µL	–	–

cobas c 501 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 24-34		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	2.8 µL	–	–

cobas c 502 **test definition**

Assay type	Rate A		
Reaction time / Assay points	10 / 24-34		
Wavelength (sub/main)	546/340 nm		
Reaction direction	Decrease		
Units	U/L (µkat/L)		
Reagent pipetting		Diluent (H_2O)	
R1	100 µL	–	
R2	20 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	2.8 µL	–	–
Decreased	1.1 µL	–	–
Increased	5.6 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

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

Traceability: This method has been standardized against the Roche system reagent 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.

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

Limitations - interference

Criterion: Recovery within ± 18 U/L of initial values of samples ≤ 180 U/L and within ± 10 % for samples > 180 U/L

Icterus:[13] 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:[13] No significant interference up to an H index of 10 (approximate hemoglobin concentration: 6.2 µmol/L or 10 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):[13] No significant interference up to an L index of 600. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

HBDH2

α-Hydroxybutyrate Dehydrogenase Gen.2

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

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. 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 required in certain cases.

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

Limits and ranges

Measuring range

6-700 U/L (0.1-11.7 µkat/L)

Determine samples having higher concentrations 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

6 U/L (0.1 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).

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 on the **cobas c** 501 analyzer.

Repeatability	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	148 (2.47)	2 (0.03)	1.0
Precipath U	260 (4.35)	2 (0.03)	0.8
Human serum 1	132 (2.21)	3 (0.05)	2.2
Human serum 2	323 (5.39)	4 (0.07)	1.2
Intermediate precision	*Mean*	*SD*	*CV*
	U/L (µkat/L)	*U/L (µkat/L)*	*%*
Precinorm U	157 (2.62)	3 (0.05)	1.9
Precipath U	259 (4.32)	4 (0.07)	1.4
Human serum 3	109 (1.82)	4 (0.07)	3.8
Human serum 4	333 (5.56)	5 (0.08)	1.5

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

α-hydroxybutyrate dehydrogenase values for human serum and plasma samples obtained on a **cobas c** 501 analyzer (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 82

Passing/Bablok[17]	Linear regression
y = 0.994x + 1.42 U/L	y = 0.995x + 2.24 U/L
τ = 0.957	r = 0.998

The sample activities were between 93.7 and 645 U/L (1.56 and 10.8 µkat/L).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

COBAS INTEGRA systems

System information

HBDH2: Test ID 0-190

Reagents - working solutions

R1 Phosphate buffer: 68 mmol/L, pH 7.5 (25 °C); α-ketobutyrate: 3.7 mmol/L; preservative

SR NADH: ≥ 1.1 mmol/L; preservative

R1 is in position A and SR is in position C.

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
On-board in use at 10-15 °C	12 weeks

Application for serum/plasma

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-S-SR
Reaction direction	Decrease
Wavelength A/B	340/409 nm
Calc. first/last	45/62
Unit	U/L

Pipetting parameters

		Diluent (H_2O)
R1	105 µL	
Sample	3 µL	10 µL
SR	21 µL	5 µL
Total volume	144 µL	

Calibration

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and 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 Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

Quality control

Reference range	Precinorm U plus or PreciControl ClinChem Multi 1
Pathological range	Precipath U plus or PreciControl ClinChem Multi 2
Control interval	24 hours recommended
Control sequence	User defined

HBDH2

α-Hydroxybutyrate Dehydrogenase Gen.2

Control after calibration Recommended

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.

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

Limitations - interference

Criterion: Recovery within ± 18 U/L of initial values of samples ≤ 180 U/L and within ± 10 % for samples > 180 U/L.

Icterus:[13] 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:[13] No significant interference up to an H index of 25 (approximate hemoglobin concentration: 16 µmol/L or 25 mg/dL).

Lipemia (Intralipid):[13] No significant interference up to an L index of 600. 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.[14, 15]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[16]

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 INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

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

Limits and ranges

Measuring range

6-700 U/L (0.10-11.7 µkat/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Lower detection limit of the test:
6 U/L (0.10 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 a zero sample (zero sample + 3 SD, repeatability, n = 21).

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 (1 aliquot per run, 1 run per day, 21 days). The following results were obtained on the COBAS INTEGRA 700 analyzer:

Repeatability	*Mean U/L (µkat/L)*	*SD U/L (µkat/L)*	*CV %*
Precinorm U	156 (2.60)	2 (0.03)	1.5
Precipath U	238 (3.97)	2 (0.03)	1.1

Intermediate precision	*Mean U/L (µkat/L)*	*SD U/L (µkat/L)*	*CV %*
Precinorm U	156 (2.60)	2 (0.03)	1.4
Precipath U	237 (3.95)	3 (0.05)	1.3

The data obtained on COBAS INTEGRA 700 analyzer(s) are representative for COBAS INTEGRA 400 analyzer(s).

Method comparison

HBDH values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer (y) were compared with those determined using the same reagent on a Roche/Hitachi 917 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 89
Passing/Bablok[17]	Linear regression
y = 0.992x - 3.60 U/L	y = 0.993x - 3.26 U/L
τ = 0.973	r = 1.00

The sample activities were between 50.3 and 637 U/L (0.840 and 10.6 µkat/L).

The data obtained on COBAS INTEGRA 700 analyzer(s) are representative for COBAS INTEGRA 400 analyzer(s).

References

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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 for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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