Cat. No 7-441

BS-400
Chemistry Analyzer

A-400 HBDH

DIAGNOSTIC KIT FOR DETERMINATION OF α-HYDROXYBUTYRATE DEHYDROGENASE ACTIVITY

INTRODUCTION
Lactate dehydrogenase (LDH, LD) is a tetrameric molecule containing two possible forms of subunits (H and M). The result is five isoenzymes, one of which is hydroxybutyrate dehydrogenase (HBDH, LD-1) formed by four H subunits. HBDH is present mainly in heart muscle but occur also in kidney and erythrocytes. Normal serum contains mostly LD-2 with lesser amount of LD-1. Changes in the ratio of LD-1 to LD-2 indicate myocardial infarction or hemolysis.

METHOD PRINCIPLE
Kinetic method of Deutsche Gesselschaft für Klinische Chemie (DGKC).

$$\text{2-Oxybutyrate + NADH + } H^+ \xleftrightarrow{\alpha\text{-HBDH}} \text{2-hydroxybutyrate + } NAD^+$$

The rate of absorbance changing at λ=340 nm is directly proportional to α-hydroxybutyrate dehydrogenase activity.

REAGENTS
Package

1-Reagent	2 x 30 ml
2-Reagent	1 x 15.5 ml

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 12 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination!

Concentrations in the test

phosphate buffer (pH 7.5)	50 mmol/l
2-oxybutyrate	3 mmol/l
NADH	0.25 mmol/l

Warnings and notes
- Product for in vitro diagnostic use only.
- The reagents contain < 0.1% sodium azide as a preservative. Avoid contact with skin and mucous membranes.

SPECIMEN
Serum.
Do not use hemolyzed blood because erythrocytes contain very high HBDH activity. Do not chill or freeze samples.
HBDH activity is unstable and is rapidly lost during storage. Specimens can be stored up to 6 hours at 15-25ºC.
Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE
These reagents may be used in automatic analyser BS-400.
1-Reagent and 2-Reagent are ready to use.
For reagent blank deionized water is recommended.

APPLICATION
BASIC

Test information		Reagent volume	
No.	24	R1	160
Test	HBDH	R2	40
Full Name	HBDH	R3	
Std. No.	24	R4	

Sample volume

Standard	10	15	10
Increased	20	15	10
Decreased	5	15	10

Reaction Parameters

Reac. Type	Kinetic	Direction	Decrease
Pri. Wave	340	Rgt. Blank	
Sec. Wave	450	Reac. Time	50 60

Result Setup

Decimal	0.1	Slope	1
Unit	U/l	Inter	0

Judgment Criteria

Absorbance	0	0	Lin. Range	9.5	1100
Incre. Test	0		Lin. Limit	0.20	
Decre. Test	0		Subs. Limit		

□ Prozone ○ Rate ○ Antigen

Q1	0	Q2	0	Q3	0	Q4	0
PC	0			ABS	0		

CALIBRATION

Calibration

Rule	Multi-point Linear
Replicate	3
K	

Judgment Criteria

Sensitivity	Blank Abs.
Factor Diff.	Error Limit
SD	Corr. Coeff.

REFERENCE VALUES [7]

serum	37ºC
adults	< 182 U/l (< 3.04 µkat/l)

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL
For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) with each batch of samples.
For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) and LEVEL 2 (Cat. No 5-175; 5-177) is recommended. As a 0 calibrator deionized water should be used.
The calibration curve should be prepared every 12 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS
These metrological characteristics have been obtained using automatic analyser BS-400. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 9.5 U/l (0.158 µkat/l).

- **Linearity:** up to 1100 U/l (18.33 µkat/l).
  If HBDH activity in tested sample exceeds or 1100 U/l dilute the sample 10-fold with 0.9% NaCl and repeat the assay. Multiply the result by 10.

- **Specificity / Interferences**
  Haemoglobin up to 2.5 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 1000 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 10	Mean [U/l]	SD [U/l]	CV [%]
level 1	158.47	0.58	0.37
level 2	393.41	0.74	0.19

Reproducibility (day to day) n = 10	Mean [U/l]	SD [U/l]	CV [%]
level 1	156.01	3.03	1.94
level 2	437.04	7.61	1.74

- **Method comparison**

A comparison between HBDH activity determined at BS-400 (y) and at Olympus AU400 (x) using 30 samples gave following results:
y = 0.933 x + 4.1779 U/l;
R = 0.999 (R – correlation coefficient)

WASTE MANAGEMENT
Please refer to local legal requirements.

LITERATURE

1. DGKC: J. Clin. Chem. Clin. Biochem. 8, 658-660 (1970).
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5. Burtis C.A., Ashwood E.R., ed. Tietz Textbook of Clinical Chemistry, 2nd ed. Philadelphia, PA: WB Saunders, 816-8, (1994).
6. Tietz N.W., ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders, 384 (1995).
7. Dembińska-Kieć A., Naskalski J.W.: Diagnostyka laboratoryjna z elementami biochemii klinicznej, Volumed, 142-144, 781, (1998).

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MANUFACTURER

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