POINTE SCIENTIFIC, INC.
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α-HBDH Reagent Set

Intended Use
For the quantitative kinetic determination of α-hydroxybutyrate dehydrogenase (HBDH) activity in serum. For *in vitro* diagnostic use only.

Clinical Significance
Determination of HBDH is useful in the diagnosis of myocardial infarction[1]. Elevated HBDH can usually be detected within 12 hours of an infarction, with peak values being determined after 48 to 72 hours.[2] Increased serum activity can also be found in the Duchenne form of muscular dystrophy, megaloblastic anemia and widely disseminated metastases[3].

Test Summary
The enzymatic activity of serum toward 2-oxobutyrate has been shown to be associated with the *a*-globulin fractions of lactate dehydrogenase isoenzymes after electrophoretic separation.[1] This activity is termed α-hydroxybutyrate dehydrogenase although it is not actually a different specific enzyme, but a representation of the activity of the LD-1 and LD-2 isoenzymes.[4] In this assay the substrate 2-oxobutyrate is not optimized, but set at a level which provides an optimum discrimination between lactate dehydrogenase isoenzymes. This reagent is based on the recommended formulation of the German Society for Clinical Chemistry. [5,6]

Principle
$$\text{2-oxobutyrate} + \text{NADH} + \text{H}^+ \xrightarrow{\alpha\text{-HBDH}} \text{2-hydroxybutyrate} + \text{NAD}^+$$

The oxidation of NADH results in a decrease in absorbance at 340nm which is proportional to the activity of the enzyme in the sample.

Reagent Composition
(Concentrations refer to reconstituted reagent)

Phosphate buffer, pH 7.5	50mM
2-oxobutyrate	3mM
NADH	0.20mM

Reagent Preparation
Add the specified volume of deionized or distilled water indicated on the vial label. Swirl gently to dissolve.

Reagent Storage and Stability
Unopened reagent vials stored in the refrigerator at 2-8°C are stable until the expiration date on the label. Reconstituted reagent is stable for 5 days stored in the refrigerator at 2-8°C.

Precautions
1. Reagent is intended for *in vitro* diagnostic use only.
2. Do not use hemolyzed samples; erythrocytes contain a high level of the enzyme being measured in this assay.
3. Do not freeze the reconstituted reagent.
4. Do not use the reagent if the absorbance at 340nm, immediately after reconstitution, is less than 0.800.
5. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2)[7]

Specimen Collection and Storage
1. Use clear, unhemolyzed serum, separated from the clot as soon as possible after collection.
2. Plasma is not recommended since heparin, oxalate and citrate reportedly inhibit HBDH activity.[8]
3. Specimens should be collected as per NCCLS document H4-A3.[9]
4. Samples may be stored for 7 days refrigerated (2-8°C) or for two weeks frozen (–20°C) if protected from evaporation.[10]

Interferences
1. Heparin, oxalate and citrate reportedly inhibit the enzyme.[8]
2. Young et al have published a list of drugs and substances which have an effect on diagnostic assays, including the determination of HBDH.[11]
3. Hemolysis in the specimen will create falsely elevated levels of HBDH since erythrocytes contain a high level of the enzyme being measured.
4. Bilirubin to the level of 20mg/dl has been found to exhibit negligible interference in this assay.

Materials Provided
HBDH reagent in vials to be reconstituted with distilled or deionized water as described.

Materials Required but not Provided
1. Spectrophotometer capable of accurate absorbance measurements at 340nm.
2. Cuvettes with 1 cm light path.
3. Constant temperature water bath. If the assay is performed and followed in the cuvette compartment of a spectrophotometer, the cuvette compartment should be temperature-controlled.
4. Distilled or deionized water.
5. Pipettes for the measurement of water, reagent and samples.

Procedure (Automated-General)

Wavelength:	340nm
Assay Type:	Kinetic
Sample/Reagent Ratio:	1:50
Reaction Direction:	Decreasing
Temperature:	25, 30, 37°C
Lag Phase:	60 sec.
Read Time:	60 sec.

Test Procedure (Manual)
1. Bring the required volume of reagent to incubation temperature. (25°C is recommended)
2. Pipette 1.0ml of reagent into tubes labeled “Patient”, “Control”, etc.
3. Zero spectrophotometer with water at 340nm.
4. Add 0.020ml (20ul) of sample and read after 60 seconds.
5. Continue readings every 60 seconds for two minutes.
6. Determine the mean absorbance difference per minute (ΔAbs./Min.).
7. Multiply the ΔAbs./min. by 8199 to obtain result in U/L.

NOTE: For manual assays, we recommend to perform the assay at 25°C. Other temperatures may be employed for the assay, such as 30°C or 37°C. The activity will vary with changes in temperature, but the calculations will remain the same. Expected values will, however, be different at various temperatures. See “Expected Values”.

Limitations
1. Samples that exceed 1000 U/L should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.

2. Hemolysis in the specimen will create falsely elevated levels of HBDH since erythrocytes contain a high level of the enzyme being measured.

Calibration
The assay is standardized by means of the millimolar absorptivity of NADH which is 6.22 under the test conditions described.

Calculation

$$\Delta\text{Abs./Min.} \times \frac{TV \times 10^6}{6.22 \times 10^3 \times LP \times SV} = U/L$$

Where:
TV = Total volume in ml (1.02)
6.22×10^3 = molar extinction coefficient of NADH
LP = Light path in cm (1)
SV = Sample volume in ml (0.02)
The factor is then 8199. Multiply the change in absorbance/minute by this factor to obtain the U/L.

Sample Calculation
If the ΔAbs./Min. of the assay is 0.045 then:
0.045 x 8199 = 369 U/L of α-HBDH activity in the sample.

Quality Control
The validity of the reaction should be monitored by use of control serums with known normal and abnormal HBDH values. These controls should be run at least with every working shift in which HBDH assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

Expected Values[13]

25°C	30°C	37°C
68-135 U/L	82-163 U/L	96-190 U/L

It is recommended that each laboratory establish its own reference range.

Performance
1. Assay range: The reagent gives linear results over the range of 0-1000 U/L. Samples that exceed 1000 U/L should be diluted with an equal volume of saline and re-assayed. Multiply the result by two.
2. Comparison: Results obtained with this reagent (y) in 114 samples ranging in α-HBDH activity form 79 to 1049 U/L were compared with those obtained in the same samples using a reagent (x) form Reagents Applications, Inc. based on the same formulation. The correlation coefficient was 0.995 and the regression equation was y=0.90x-3.8. (Sy-x = 43.80).
3. Precision: Precision studies were performed following the guidelines which are contained in NCCLS document EP5-T2.[12]

Within Run (N=20)			Run to Run (N=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
131	4.1	3.1	134	3.1	2.3
405	6.5	1.6	404	6.6	1.6

4. Sensitivity: The sensitivity for the HBDH reagent was investigated by reading the change in absorbance per minute at 340nm for a saline sample, and a serum with a known concentration. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the HBDH reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 U/L HBDH activity gives an ΔAbs./Min of 0.0001.

References
1. Rosalki, S.B. and Wilikinson, J.H., Nature 188:1110 (1960).
2. Rosalki, S.B. and Wilkinson, J.H., JAMA 189:64 (1965).
3. Wilkinson, J.H., Methods of Enzymatic Analysis, Vol.2, New York, Academic Press, pp.603:612 (1974).
4. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders Company, p.659 (1982).
5. Z. Klin. Chem. U. Klin. Biochem. 8:658 (1970).
6. Z. Klin. Chem. U. Klin. Biochem. 10:182 (1972).
7. NCCLS document "Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue", 2nd Ed. (1991).
8. Friedel, R. and Mattenheimer, H., Clin. Chim. Acta 61:F-11 (1975).
9. NCCLS document "Procedures for the Collection of Diagnostic Blood Specimens by Skin Puncture", 3rd Ed. (1991).
10. Rosalki, S.B., J. Clin. Pathol. 15:556 (1962).
11. Young, D.S., Pestaner, L.C. and Gibberman, V., Clin. Chem. 21:5 (1975).
12. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).
13. Weisshaar, D., et al., Med. Welt 9:387 (1975).

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