LACTATE DEHYDROGENASE (LDH)

DGKC Method

Diagnostic reagent for determination of LDH concentration.
Liquid. Dual reagent. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Pack
MH-482	75 mL
MH-483	50 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The test is applied for the quantitative determination of LDH in serum and plasma.

GENERAL INFORMATION

Lactate dehydrogenase (EC 1.1.1.27; L-lactate: NAD^+ oxido-reductase; LDH) is a hydrogen transfer enzyme that catalyzes the oxidation of L-lactate to pyruvate via NAD^+ as a hydrogen acceptor. The reaction is reversible and the reaction equilibrium strongly favors the reduction of pyruvate to lactate (P→L) (reverse reaction). The optimum pH for the reaction of pyruvate formation from lactate substrate (L→P) is pH: 8.8-9.8 (Figure 1).[1]

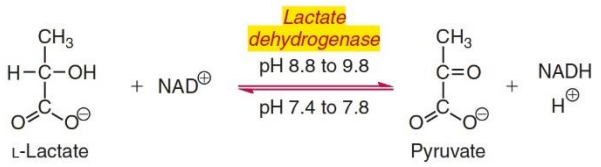

Figure 1. *Reversible oxidation-reduction reaction catalyzed by the enzyme lactate dehydrogenase* [1]

The enzyme has a molecular weight of 134,000 D and consists of four peptide chains of two types, M (or A) and H (or B), each under separate genetic control. As a result, there are 5 isoenzymes: H4 (named LDH1 because of its electrophoretic mobility towards the anode), H3M1 (LDH2), H2M2 (LDH3), H1M3 (LDH4) and M4 (LDH5). A distinct 6th LDH isoenzyme, LD-X (also called LDc), composed of four X (or C) subunits, is present in human testes after puberty. A seventh LDH, called LDH6, has been detected in the serum of severely ill patients. The most common method used to separate LDH isoenzymes is electrophoresis.[2]

LDH activity is present in all cells of the body and is always found only in the cytoplasm of the cell. Concentrations of the enzyme in various tissues are approximately 1500 to 5000 times higher than those physiologically present in serum.

Therefore, leakage of the enzyme from even a small mass of damaged tissue significantly increases the observed serum activity of LDH. Different tissues show different isoenzyme composition. LDH1 and LDH2 predominate in the heart, kidneys (cortex) and erythrocytes, whereas LDH4 and LDH5 isoenzymes predominate in liver and skeletal muscle.[3]

Because of its wide spread in all tissues, increases in serum LDH activity occur in a variety of clinical conditions, including myocardial infarction, hepatitis, hemolysis, and renal, pulmonary and muscular disorders. A systematic review of the literature shows that the serum LDH analyte is only relevant in hematology and oncology.[4]

Hemolytic anemias significantly increase LDH concentrations in serum. Significant increases in LDH activity up to 50 times the upper reference limit (URL) have been observed in megaloblastic anemias. These anemias cause the breakdown of erythrocyte precursor cells in the bone marrow (ineffective erythropoiesis), resulting in the release of large amounts of LDH1 and LDH2 isoenzymes. These elevations rapidly return to normal after appropriate treatment. For monitoring purposes, LDH levels are relevant for estimating the survival rate (probability of survival) and duration of Hodgkin's disease and non-Hodgkin's lymphoma.[2]

Patients with malignant disease often show increased LDH activity in serum. Up to 70% of patients with liver metastases and 20% to 60% of patients with other non-hepatic metastases have increased total LDH activity. Significantly increased LDH is observed in germ cell tumors (60% of cases) such as teratoma, testicular seminoma and ovarian dysgerminoma.[5] LDH appears to be a useful predictor of outcome in patients with testicular germ cell tumors, melanoma and small cell lung cancer.[2] Increases in LDH activity (predominant LDH-4 and LDH-5 isoenzymes) are observed in liver disease, but their clinical utility in liver profiling appears to be limited and they do not appear to contribute significantly to the investigation of aminotransferase activity.[1]
LDH measured in pleural fluid (in combination with serum LDH) helps differentiate between exudative effusions and transudative effusions.[6]

Macro-LDH, which results from the formation of an autoantibody-enzyme complex leading to a persistent increase in the amount of circulating enzyme up to eight times the URL, is present in less than 1/10,000 individuals. Documentation of a macro-LDH in suspected individuals (e.g. the presence of an abnormally displaced band on electrophoresis) should be provided to avoid additional follow-up, investigations or unnecessary treatment.[1]

TEST PRINCIPLE

Kinetic, UV spectrophotometric method

In the reduction reaction catalyzed by LDH, NADH participates as hydrogen donor with pyruvate as substrate. After this reverse reaction, NAD^+ is formed as a product along with lactate. The change in absorbance measured at 340 nm during the NADH/NAD^+ conversion is proportional to LDH activity.

Annotation:

- An optimized L→P reference method for LDH1 has been developed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).[7] This method has recently formed the basis for the development of an IFCC primary reference procedure for LDH at 37°C.[8] Electrophoretic separation using agarose gel or cellulose acetate membranes is the most widely used procedure to demonstrate LDH isoenzymes.[9]

REAGENT COMPONENTS

Phosphate buffer : ≤60 mmol/L
Sodium pyruvate : ≤70 mmol/L
NADH : ≤20 mmol/L

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[10]

SAMPLE REQUIREMENTS

Serum or plasma collected by standard procedure must be used. Li-heparin collection tubes must be preferred for plasma. Multiple sample freezing and thawing should be avoided.

LDH activity stability in serum and plasma[24,25]:

3 days at +20/+25ºC
3 days at +2/+8ºC
8 weeks at -20°C

Annotation:

- Hemolyzed samples should not be used.
- Serum is the preferred sample for measuring LDH activity. Plasma samples may be contaminated with platelets containing high concentrations of LDH.[11]
- Different isoenzymes differ in their sensitivity to cold, in particular LDH4 and LDH5 are characterized as labile in cold. The activity of LDH4 and LDH5 is lost if samples are stored at -20°C. Therefore, when necessary, serum samples can be stored at room temperature where no loss of activity is likely to occur for at least up to 3 days.[1,2]
- It is possible that EDTA inhibits the enzyme by binding Zn^{+2}, which has been suggested to be an activator of LDH.[1]

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a Arcal Auto Calibrator.

Arcal Auto Calibrator-Lyophilized
Ref. No: VT-003

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Arcon N Level 1 Control- Lyophilized
Ref.No: VT-001

Arcon P Level 2 Control- Lyophilized
Ref.No: VT-002

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Adults[26]: 230 - 460 U/L

Values of LDH activity in serum vary considerably depending on the direction of the enzyme reaction and the method used.[2]
The reference interval in white adult subjects determined by a traceable assay according to the IFCC reference procedure at 37°C has been found to be 125 to 220 U/L.[12]

LDH reference limits are higher in children (180 to 360 U/L).[13]

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

Reference interval has been verified by using CLSI EP28-A3c protocol.[14]

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

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or

any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[15]

The determined analytic measuring interval for LDH is 30–2750 U/L.

Detection Capability

Limit of Detection (LoD): 5 U/L

Limit of Quantitation (LoQ): 30 U/L

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[16]

Linearity

This method shows measurement linearity in the activities up to 2750 U/L. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:5 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.
Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[17]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatibility and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.
According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[18]

Repeatibility (Within Run) and Repeatibility (Day to Day) SD (standard deviation) and CV% values of LDH have been given in the table 1 and 2 respectively.

Table 1. LDH Repeatibility (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
250 U/L	4.28	1.71	80
721 U/L	9.38	1.30	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[19]

Table 2. LDH Repeatibility (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
250 U/L	7.69	3.08	80
721 U/L	18.52	2.57	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[19]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablok equation:[20]
y= 0.99x + 2.41 U/L
r= 0.99

Interference

Endogenous interferant and analyte concentrations that have been used in the LDH scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[21,22]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from LDH interference scanning test is appropriate, is determined as ±10%.[23]

In LDH test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	LDH Target (U/L)	N*	Observed Recovery %
Lipemia 2149 mg/dL	247	3	104

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).[22]*

Annotation:

- The P→L reaction is more dependent on NAD+ and lactate concentrations than the L→P reaction, and there may be more contamination of NAD^+ with inhibitory products.[1,7,8]
- Hemolyzed serum should not be used because erythrocytes contain 4000 times more LDH activity than serum.[1]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic

antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[22]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD**:** For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests

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