Bialex®

# LDH

## CLINICAL SIGNIFICANCE

**Physiological Distribution and Specificity.** Lactate Dehydrogenase (LDH; EC 1.1.1.27) is an essential cytoplasmic enzyme found in almost all living cells. Its highest concentrations are located in organs with high metabolic turnover, such as the heart, liver, skeletal muscle, kidneys, and lungs, as well as in erythrocytes. Within the cell, LDH acts as a catalyst for the reversible interconversion of pyruvate and lactate. This step is crucial for anaerobic glycolysis, allowing cells to maintain energy production (ATP) when oxygen supply is limited. Regarding its **specificity**, LDH is considered a marker of low organ-specificity because it is present in so many different tissues. However, its clinical value lies in its high **sensitivity** to cellular stress. Under normal conditions, the concentration of LDH inside the cells is approximately 500 times greater than in the extracellular space. Consequently, even minor cell membrane damage or necrosis results in a significant and measurable increase in serum LDH activity, making it an excellent "universal" indicator of tissue injury.

**Diagnostic Utility.** The measurement of LDH activity is a fundamental tool in clinical diagnostics and patient monitoring across several medical fields. Due to its broad tissue distribution, it is utilized as follows:

- **Tissue Damage Marker:** It serves as a general indicator of acute or chronic tissue injury. It is particularly useful in the diagnosis of **myocardial infarction** (where levels rise 12–24 hours after the onset of pain), pulmonary embolism, and renal infarction.
- **Hematology:** LDH is a critical marker for **hemolytic anemias** (such as megaloblastic or sickle cell anemia) and other hematologic disorders like leukemia and lymphoma, where high cell turnover significantly increases serum activity.
- **Oncology:** In cancer patients, elevated LDH levels are often associated with tumor mass, growth rate, and poor prognosis. It is routinely used to monitor the effectiveness of chemotherapy and disease progression in germ cell tumors and various carcinomas.

- **Hepatobiliary Disease:** Although less specific than transaminases, LDH levels aid in the differential diagnosis of liver diseases, including toxic hepatitis and infectious mononucleosis.
- **Body Fluids Analysis:** The determination of LDH in cerebrospinal fluid (CSF) or pleural/pericardial effusions helps differentiate between transudates and exudates (Light's criteria), providing vital clues for infectious or inflammatory etiologies.

# METHOD PRINCIPLE

The determination of Lactate Dehydrogenase (LDH) activity is based on the methodology recommended by the **International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)**.

The method measures the rate of the enzymatic reaction where LDH catalyzes the reduction of pyruvate to lactate, coupled with the simultaneous oxidation of the coenzyme NADH to NAD+.

## Reagents Composition

| Name | Qty | Values |
|---|---|---|
| Tris Buffer, | 100 mMol/L | pH 7.2-7.7 |
| Piruvato | 100 mMol/L | NRD |
| NADH | 2 mMol/L | NRD |
| Preservative | <1% | NRD |

**Reagents Provided:** 02 vial reagents **(Ref: GQ-LDH-100, GQ-LDH-125)**

## Materials Required but not provided

- Clinical Chemistry Analyzer (Full, Semi-automated) with 37°C temperature control and filter of 340nm
- Deionized water and Class A glassware.
- Automatic Pipettes
- Assayed Human Control serum (Optional, for validation)
- PassCheck Calibrator (GQ-CAL-5)

| Sample Collection | Interference | Storage and Stability of Reagent | ASSAY PRECAUTIONS |
|---|---|---|---|
| • **Serum:** Use standard collection tubes. Allow the blood to clot completely and centrifuge as soon as possible.<br>• **Plasma:** Use **Heparin** as an anticoagulant.<br>• *Note:* Do **not** use EDTA, Oxalate, or Citrate, as they may interfere with enzyme stability or the reaction environment.<br>• **Cerebrospinal Fluid (CSF):** Collect in a sterile container. Analyze immediately to prevent cell lysis and protein degradation. | • **Hemolysis (Major Interference):** As previously stated, erythrocytes contain LDH concentrations approximately 500 times higher than serum. Any degree of hemolysis will lead to **falsely elevated results**. Even samples with non-visible hemolysis can show significant increases.<br>• **Bilirubin:** No significant interference has been observed up to **40 mg/dL** of bilirubin.<br>• **Lipemia (Turbidity):** High concentrations of triglycerides (> 600 mg/dL) may interfere with the optical reading at 340 nm. It is recommended to centrifuge lipemic samples at high speed or use a sample blank if necessary.<br>• **Ascorbic Acid:** No significant interference up to **30 mg/dL**.<br>• **Anticoagulants:** As mentioned in the sample collection section, **EDTA, Oxalate, and Citrate** act as inhibitors and must be avoided. Only Heparin is recommended for plasma samples.<br>• **Drugs and Toxins:** Certain therapeutic drugs and substances may affect LDH activity in vivo or in vitro. (e.g., Young DS, *Effects of Drugs on Clinical Laboratory Tests*). | • Store at **2-8°C** protected from light.<br>• The reagent is stable until the expiration date indicated if uncontaminated.<br>• **On-Board Stability (Separate R1 & R2): 30 days**, provided the reagents are kept in the refrigerated compartment (2 to 8°C) **(Work reagent): 14 days** when stored refrigerated. **(Work reagent at room temperature):** 24 hours (20-25°C) | 1. For *in vitro* diagnostic use only.<br>2. Reagent contains Sodium Azide (0.09%) as a preservative.<br>3. **Optical Absorbance: The initial absorbance** of the reagent (or the mixture R1+R2) is **lower than 1.000** at 340 nm (1 cm light path). This indicates a significant degradation of the NADH coenzyme.<br>4. **Physical Appearance:** Presence of turbidity, particles, or visible microbial growth in either R1 or R2.<br>5. **Calibration Failure:** Inability to recover the expected values for commercial control serums. |

## Work Reagent Preparation:

Dilute 250 µL of substrate into 1 mL of buffer (1:4). Homogenize gently and allow to stand for 3 minutes at 37°C before use. The working reagent is stable for up to 7 days at room temperature.

## Instrument Assay Procedure

| Semi Automatic | Automatic |
|---|---|
| • **Method:** Rate Method<br>• **Calibration Type:** Factor Method<br>• **Calibration Point 1:** 8095<br>• **Wavelength:** 340 nm.<br>• **Temperature:** 37°C.<br>• **Blank Setting:** DI Water<br>• **Reagent Volume:** 500 µL.<br>• **Sample Volume:** 10 µL.<br>• **Lag Time:** 60 seconds.<br>• **Read Time:** 120 seconds<br>• Range: 100 - 230<br>• Linear: 1-1600<br>• Bring reagent and samples to Room Temperature 25°C | • **Reaction Type:** Decreasing<br>• **Wavelength:** 340 nm.<br>• **Temperature:** 37°C.<br>• **Blank Setting:** DI Water<br>• **Reagent Volume:** 250 µL.<br>• **Sample Volume:** 5 µL.<br>• **Lag Time:** 60 seconds.<br>• **Read Time:** 120 seconds.<br>• **Factor:** Please refer to the Lot-specific Certificate of Analysis for the appropiate calibration factor.<br>• **Reagent Onbord:** 3 Weeks - Refrigerated |

# OTHER REFERENCES

| Performance | Reference Values | Common errors | BIBLIOGRAPHY |
|---|---|---|---|
| • **Limit of Detection:** 1 U/L (Instrument dependent).<br>• **Linearity:** Up to 1600 U/L (Dilute 1:10 for higher samples than 1000U/L).<br>• **Precision:** Intra-assay C.V. < 0.99%.<br>• Correlation coefficient (r): 0.998<br>• Regretion equation:<br><br>y = 1.012x + 1.65 | **Serum or Plasma:**<br><br>Adults 135-225 U/L<br><br>Children 110-300 U/L<br><br>Infant 225-600 U/L<br><br>Newborns (first days) 425-950 U/L<br><br>**CSF:**<br><br>Adults < 40 U/L<br><br>Neonates < 70 U/L | **Check List:**<br><br>• **Hemolyzed Samples**<br>• **Bubbles in the cuvettes or flow cell:** As this is a light-scattering method, bubbles will falsely elevate results.<br>• **Cuvettes Opaque or dirty :** Residues from other reagents containing detergents will inhibit coenzyme activity.<br>• **Unstable temperature:** Enzymatic kinetics vary by approximately 7% per degree Celsius.<br>• **Electronic Noise:** Faulty electrical impedance or poor grounding can destabilize absorbance readings at 340 nm, leading to erratic kinetic signals and inaccurate activity results. | 1. **Young DS.** *Effects of Drugs on Clinical Laboratory Tests.* 5th ed. AACC Press, 2000.<br>2. **Burtis CA, Ashwood ER, Bruns DE.** *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.* 5th ed. Elsevier Saunders, 2012.<br>3. **Ziegler M, et al.** *Stability of Lactate Dehydrogenase in Serum and Plasma.* Clin. Chem. 1998; 44: 1785-1786. |

# QUALITY CONTROL

**In-House Performance Validation:**

The performance of this LDH reagent has been validated internally using an automated analyzer and certified commercial

**System Validation and Calibration:**

**Calibration**

The assay can be performed using either a **theoretical factor** or

**Intra-Laboratory Quality Program:**

To ensure the long-term reliability of results, each laboratory must implement an internal quality control

control materials. The following data represent the results of our internal quality control (QC) assessment. **On-Board Stability and Method Comparison**

A study was conducted to evaluate the reagent's consistency during routine laboratory operation. A total of **50 clinical samples** were analyzed over a continuous period.

- **Observed Variation:** The assay demonstrated a cumulative variation of **±2%** across the clinical range.
- **Correlation:** Despite the observed deviation, the method maintains a strong correlation with the initial calibration values and clinical expectations.
- **Conclusion:** The reagent remains functional and reliable for routine use. However, for maximum clinical precision, **daily calibration** or the use of daily control a **calibrator-based method**:

- **Factor Calculation:** In most automated and semi-automated systems (flow cell), the activity is calculated using the technical factor of **8095** (at 340 nm, 37°C, with a 1:51 dilution).
- **Calibration with Standard:** It is recommended to use a certified **Clinical Chemistry Calibrator** (e.g., PassCheck Multicalibrator). The instrument should be calibrated:
  - When changing the reagent lot.
  - After significant maintenance of the instrument (e.g., flow cell cleaning or lamp replacement).

program. This program should include:

- **Daily Monitoring:** Analysis of at least two levels of control materials (Normal and Pathological) every 24 hours or with each batch of samples.
- **Trend Analysis:** Results should be plotted on **Levey-Jennings charts** to monitor the precision of the assay. The application of **Westgard Rules** is recommended to identify systematic errors (shifts) or random errors (imprecision).
- **Reagent Blank Tracking:** The initial absorbance of the Reagent Blank should be recorded daily. A sudden drop in absorbance indicates reagent degradation or light exposure.
- **Acceptability Criteria:** The laboratory should

serums is recommended to compensate for the ± 2% operational drift.

- When quality control results fall outside the established limits.

- **System Validation (Quality Control)** To ensure daily system performance, the use of **Control Serums** (Normal and Pathological) is mandatory:
  - At least two levels of control should be analyzed at the beginning of each work shift.
  - Results must fall within the specific range established by the laboratory.
  - If control values are outside the expected range, patient results should not be reported until the system is re-validated (check temperature, reagent blank,

define its own "Total Allowable Error" (TEa) based on clinical requirements. If results exceed the established standard deviation (SD), a full system review (calibration, temperature, and flow cell cleanliness) must be performed.

and flow cell
cleanliness).

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