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Lactate Dehydrogenase: Analytical Aspects

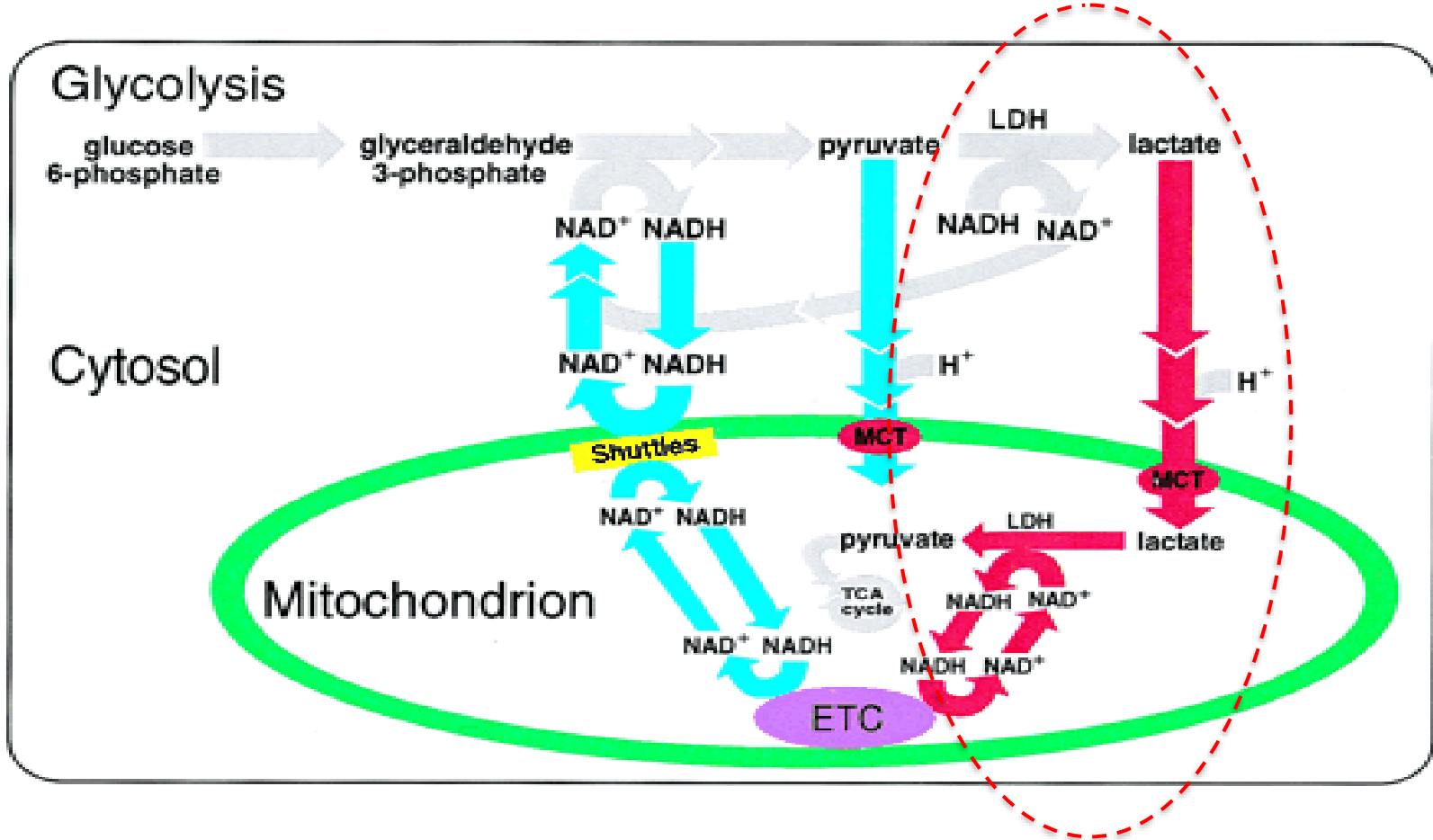
Chesinta Voma, PhD.

University of Louisville Hospital

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



Lactate Dehydrogenase (LD): A Ubiquitous Enzyme



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LD Isoenzymes

Isoenzymes	Subunit Composition	Tissue Distribution	Approx. % in Serum
LD-1	H ₄ 	Heart , RBCs, Kidney	17 – 27%
LD-2	H ₃ M ₁ 	Heart, RBCs, Kidney	27 – 37%
LD-3	H ₂ M ₂ 	Lungs	18 – 25%
LD-4	H ₁ M ₃ 	Liver, Muscle	8 – 16 %
LD-5	M ₄ 	Liver, Skeletal Muscle	6 – 16%

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Pathological Diseases Related to LD Testing

Blood (Plasma / Serum)	Body Fluids	Urine
Cardiac and hepatic diseases (3)	Differentiate bacterial vs. viral meningitis (5,6)	Malignant prostate tumor (7)
Tumors of the lung and kidney (4)	Viral meningitis: - CSF Lactate \leq 3 mmol/L Acute bacterial meningitis: - CSF Lactate \geq 6 mmol/L	Bladder malignancies (3)



Clinical Utility of Measuring LD Isoenzymes

- Limited utility today
- Ascitic LD isoenzyme pattern observed in:
 - Cirrhosis
 - Spontaneous bacterial peritonitis
 - Congestive heart failure
 - Tuberculosis
 - Malignancy



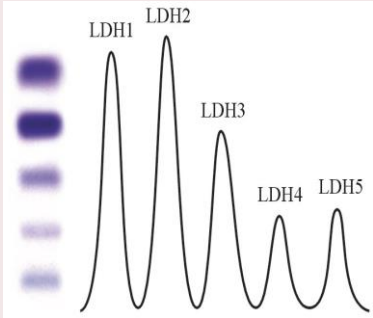
Specimen Collection: Points to Remember

Compatible Anticoagulants	Incompatible Anticoagulants
Ammonium Heparin	EDTA For the reverse reaction: Pyruvate -----→ Lactate
Lithium Heparin	Potassium Oxalate
Sodium Heparin	Sodium Citrate

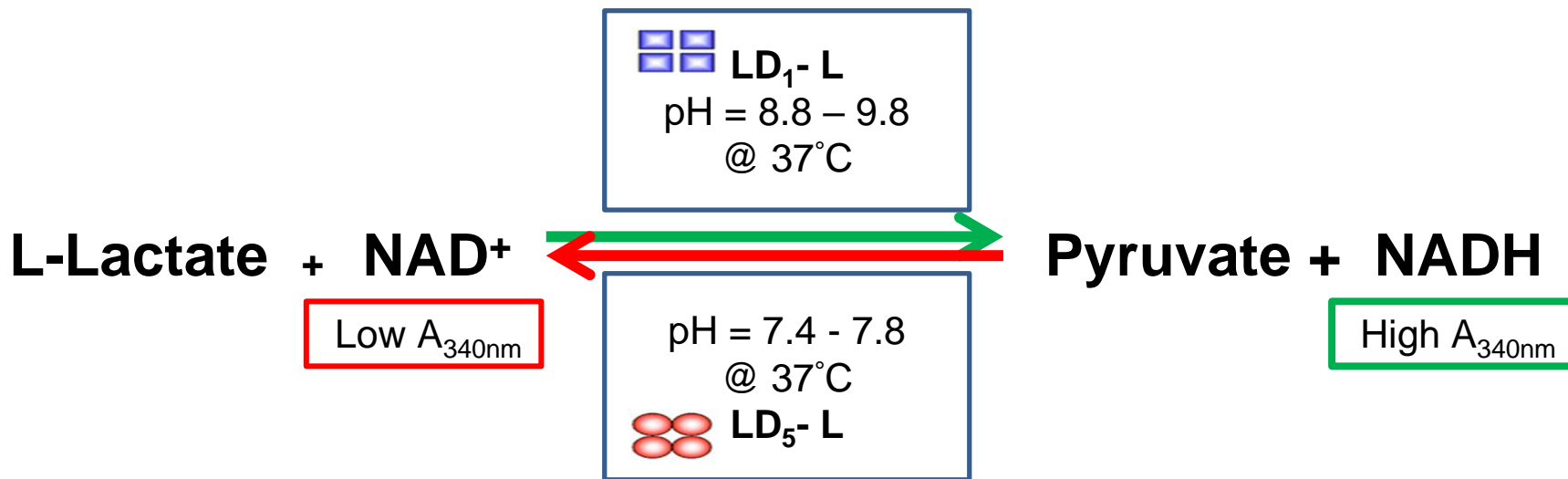
***LD is extremely sensitive to hemolysis. LD may be falsely elevated due to in vivo or in vitro hemolysis or in patients with elevated platelet counts**



Analytical Methods to Quantitate LD

Analyte Measured	Method	Advantages	Disadvantages
Total LD	Kinetic (10)	<ul style="list-style-type: none"> Speed, Inexpensive Adaptable to automation 	Unable to quantitate isoenzymes, if needed
LD Isoenzymes	Electrophoresis (10) 	<ul style="list-style-type: none"> Patterns are directly observable All isoenzymes are resolved in a single procedure 	Time-consuming

Measurement of Total LD: Kinetic Method



Forward or Reverse Kinetic Reaction?

Forward Reaction: Lactate -----→ Pyruvate	Reverse Reaction: Pyruvate -----→ Lactate
Used by 3,589 laboratories (2015 CAP Survey Data)	Used by 629 laboratories (2015 CAP Survey Data)
Lactate is a more specific substrate for the enzyme	Pyruvate is less specific, serves as a substrate for pyruvate dehydrogenase
Most commonly used in electrophoretic separation of isoenzymes, allows detection of fluorescent NADH	Less commonly used in electrophoretic separation

***Forward reaction is preferred and recommended by the IFCC**



Enzyme Activity and Michaelis-Menten Kinetics



- LD-1: high affinity for lactate, allosterically inhibited by high levels of pyruvate

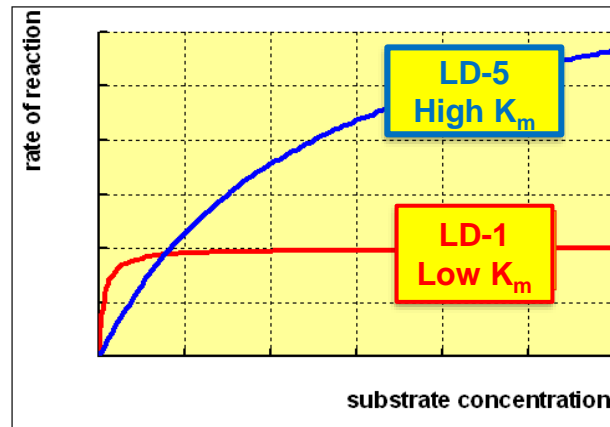


- LD-5: low affinity (higher K_m) but high capacity for pyruvate, not allosterically inhibited



- LD-2, 3, and 4: intermediate activities

Enzyme Kinetics



Stability of LD

- LD isoenzymes are more stable at room temperature (7)
- NAD⁺ or GSH content increases sera stability
- Should be stored at -90°C for long-term storage (12)
- Freeze/thaw cycles should be avoided (12)



Summary

- LD is a ubiquitous glycolytic enzyme
- Predominantly intracellular, primarily utilized as a marker of cellular & tissue damage
- Catalyzes the reversible oxidation of lactate to pyruvate
- Enzyme activity is based on differences in catalytic properties and structural stability
- LD activity can be measured using either the forward reaction (i.e., increase A340) or reverse reaction (i.e., decrease A340)



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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

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