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TITLE: Lactate Dehydrogenase: Analytical Aspects

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Slide 1:
Hello, my name is Chesie Voma. I am a Clinical Chemistry Fellow at the University of Louisville Fellowship Program. Welcome to this Pearl of Laboratory Medicine on "Lactate Dehydrogenase: Analytical Aspects."

Slide 2: Lactate Dehydrogenase (LD): A Ubiquitous Enzyme
Lactate dehydrogenase (abbreviated as LD) is ubiquitous in the cytoplasm of almost all human body cells and many other organisms, and is also present in the mitochondria of some human cells such as astrocytes of the central nervous system. In glycolysis, LD catalyzes the reversible conversion of pyruvate to lactate which is essential for glycolytic metabolism under anaerobic conditions (e.g. Cori cycle) and glucose synthesis.

Clinically, LD is important as a non-specific marker of cellular and tissue inflammation and damage.

Slide 3: LD Isoenzymes
There are 5 isoenzymes of LD, composed of different combinations of subunits (H and M) within the tetrameric structure.

LD-1 and LD-2 are present in the heart, Red Blood Cells, and kidneys. LD-3 is primarily in the lungs and LD-4 and LD-5 are present in liver and muscle tissue. Plasma concentrations of each isoenzyme varies, but the majority of circulating LD in normal, healthy individuals is LD-2. Determination of isoenzyme patterns in plasma or serum may provide useful information about the affected tissue or particular pathology, but LD isoenzyme testing is no longer a routine test.

Slide 4: Pathological Diseases Related to LD Testing
LD is an intracellular enzyme; therefore, only be a small amount of detectable LD is quantitated in the extracellular compartment of plasma or serum in normal individuals. This difference forms the basis of its utility as a marker of non-specific cellular damage.

When plasma or serum LD concentrations are above the upper limit of normal, it is an indicator of acute or chronic tissue inflammation or damage, usually from the liver, skeletal muscle, or kidneys. The absolute LD concentration also helps assess the severity of the damage, i.e. higher concentrations correlate with greater damage.

LD can also be measured in body fluids such as cerebrospinal fluid (CSF), where it can be used to differentiate viral from bacterial meningitis and also used as a prognostic indicator in patients with lymphoma.

Slide 5: Clinical Utility of Measuring LD Isoenzymes

Compared to measurement of total LD, there are fewer clinical scenarios where isoenzyme analysis is beneficial. Testing of LD isoenzymes may be useful in the differential diagnosis of the most common causes of ascites including: cirrhosis, spontaneous bacterial peritonitis, congestive heart failure, tuberculosis, and malignancy.

Slide 6: Specimen Collection: Points to Remember

Serum and heparinized plasma are both acceptable specimen types to measure LD. Use of EDTA plasma is not recommended because EDTA inhibits the actual analytical reaction used to quantitate LD. Potassium oxalate plasma is also not a recommended specimen type because it competes with lactate for binding sites.

Besides having the right sample type and anticoagulant, LD is extremely sensitive to hemolysis and elevated platelet counts, both of which yield false elevations.

Slide 7: Analytical Methods to Quantitate LD

Kinetic methods are frequently used to measure total LD. They are beneficial due to the adaptability on high throughput, automated instruments. Therefore, total LD can be easily measured and reported in a rapid manner with relatively low cost. LD isoenzymes are not frequently ordered and have not been adapted to automated instrumentation.

Electrophoresis is also used to analyze LD isoenzymes. The method and procedure are time-consuming and fairly manual but allows for direct observation of all the isoenzymes in a single procedure.

Slide 8: Measurement of Total LD: The Kinetic Method

Kinetic methods to measure total LD quantitate the change in absorbance at multiple wavelengths over a fixed period of time. LD present in the patient sample is used to catalyze the reaction of lactate and pyruvate. It is possible to measure the conversion from either direction.

The forward reaction is the one goes from left to right, converting lactate to pyruvate. The International Federation of Clinical Chemistry (IFCC) has defined the specific conditions for the forward reaction (pH, substrate concentrations, temperature, etc.).

The reverse reaction moves from right to left, converting pyruvate to lactate. The reverse reaction is performed at a physiological pH and has a greater affinity for LD-5. This becomes relevant if there is a significant delay between specimen collection and analysis because LD-5 is the least stable of the isoenzymes. In this scenario, the reverse reaction may underestimate the total LD; therefore, the forward reaction is the preferred method.

Slide 9: Forward or Reverse Kinetic Reaction?

Approximately 3600 of labs participating in the 2015 CAP proficiency testing survey for LD use the forward reaction, while 629 labs report methods which use the reverse reaction. There are several reasons why the forward reaction is the preferred way to measure total LD. First, the IFCC has defined appropriate requirements for the assay and reaction, which allows for harmonization of results between labs. Second, lactate is the preferred substrate for the LD isoenzymes while pyruvate also serves as a substrate for pyruvate dehydrogenase (PDH).

However, the reverse reaction does have some benefits. The equilibrium constant is large so the rate of reaction can be 2 to 3 times faster compared to the forward reaction. This allows smaller sample volumes to be used and shorter reaction observation times which may potentially result in a shorter turnaround time.

The forward reaction is also the most commonly used visualization technique in isoenzyme electrophoresis because it allows fluorescent NADH to be detected. However, this reason alone is not sufficient to choose a forward reaction method.

Slide 10: Enzyme Activity & Michaelis-Menten Kinetics

Each of the isoenzymes have different affinities for lactate. LD-1 has the highest affinity for lactate and is allosterically inhibited by high levels of pyruvate, whereas LD-5 has the lowest affinity (higher Km) but a high affinity for pyruvate and is not allosterically inhibited. LD-2, 3, and 4 have intermediate activities. These affinities are important to remember if the total LD results do not fit the clinical picture, but more often than not, hemolyzed samples are to blame for inconsistencies between results and patient presentation.

Slide 11: Stability of LD

There are several factors that impact the stability of LD. The isoenzymes are susceptible to freeze-thaw induced denaturation and the isoenzymes are actually more stable at room temperature than at 4 degrees Celsius. Addition of NAD or GSH to the sample will improve stability, but this is rarely utilized. While the loss of LD-5, which is the least stable, represents a small portion of the total LD in samples from healthy individuals, those with liver- or skeletal muscle-related LD elevations will have a greater portion of the total made up of LD-5; thus, analysis may underestimate the total if samples are not analyzed in a timely manner. If a laboratory routinely analyzes total LD on samples, it should adjust its reference intervals to reflect the population and impact on the methodology.

Slide 12: Summary
In summary, LD is a ubiquitous cytosolic glycolytic enzyme which catalyzes the reversible oxidation of lactate to pyruvate in glycolysis. Because of the high concentrations found intracellularly, it has utility as a marker of cellular and tissue damage. LD activity can be measured using either the forward reaction (i.e., increase A340) or reverse reaction (i.e., decrease A340).

Slide 13: References

Slide 14: Disclosures

Slide 15: Thank You from www.TraineeCouncil.org
Thank you for joining me, Chesie Voma, on this Pearl of Laboratory Medicine on “Lactate Dehydrogenase: Analytical Aspects.”