Further Testing Required:

1. In all clinical situations, LD activity may be elevated secondary to many conditions. The severity of the elevation will vary according to the condition.
2. LD elevation has not been associated with any particular pathology. LD elevation may be secondary to many conditions, including trauma, infection, etc.
3. Haptoglobin studies should be performed to rule out hemolysis as a potential cause of the elevation.
4. Myocardial infarction is the most common cause of LD elevation. The most cathodic is LD5, found primarily in liver and skeletal muscle. The others - LD2, LD3 and LD4 - are found in varying degrees of the lymphatic system such as infectious mononucleosis, lymphomas and neoplastic diseases. It is also increased in many types of liver injuries such as hepatitis and cirrhosis. LD elevation may be secondary to many conditions, including trauma, infection, etc.

Fraction Mean SD CV
LD1 5.7 0.2 3.6% 
LD2 32.9 0.6 1.9 
LD3 22.5 0.2 0.8 
LD4 6.7 0.5 7.0 
LD5 9.9 0.4 3.5 

**LINEARITY**

Fraction Mean SD CV

**SENSITIVITY**

Between Runs: Both spectrophotometers were analyzed in replicate on eight gels using the same reagents and the same gel concentration. Correlation coefficients were calculated for both machines.

**CAUTION**

The diluent should be stored at 2 to 8°C, and is stable until the expiration date on the vial and box. The diluent is stable at room temperature for at least 1 month. The QuickGel LD kit is a stabilized reagent.

The QuickGel LD kit is designed for use with QuickGel Chambers, and the QuickGel LD 2000 and 3000 systems. The QuickGel LD kit is not intended for use with any other system.

**INSTRUCTIONS FOR USE**

1. Serum is the specimen of choice. Plasma from blood specimens should be analyzed as soon after collection as possible. Serum samples should be stored at 2 to 8°C until analysis.

2. Filter the specimen before analysis:

   a. Blood samples should be centrifuged to remove red cells and platelets.
   b. Plasma should be centrifuged to remove red cells and platelets.
   c. Serum should be centrifuged to remove red cells and platelets.

3. Aliquots of the sample should be analyzed in duplicate or triplicate.

4. The QuickGel LD kit is designed for use with QuickGel Chambers and the QuickGel LD 2000 and 3000 systems. The QuickGel LD kit is not intended for use with any other system.

**SCIENTIFIC DATA**

The QuickGel LD kit has been shown to have a high degree of precision and accuracy. The method is based on the principles of electrophoresis and enzymatic analysis. The QuickGel LD kit is designed for use with QuickGel Chambers and the QuickGel LD 2000 and 3000 systems. The QuickGel LD kit is not intended for use with any other system.

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**SUGGESTED RECORDS**

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I. Introduction

II. Sample Preparation

A. Preparation of the Serum Samples

B. Preparation of the Reference Samples

C. Precautions

III. Sample Template Application

A. Template Application Procedure

B. Template Results

C. Precautions

IV. Electrophoresis and Incubation

A. Electrophoresis Procedure

B. Incubation Procedure

V. Preparation of Reagent

VI. Electrode Supply/Visualization

A. Electrode Supply

B. Electrode Visualization

VII. Sample Application

A. Sample Application Procedure

B. Sample Application Results

C. Precautions

VIII. Evaluation of LD Isoenzyme Bands

IX. Calculation of the Unknown

X. Conclusion

xi. Evaluation of LD Isoenzyme Bands

xii. Calculation of the Unknown

xiii. Conclusion

xiv. References

xv. Abbreviations

xvi. Color Plates
II. Chamber Preparation

LD Reagent (10 x 1.0 mL)

Materials Provided:

- SPIFE Applicator Blades 3450
- QuickGel Gel Block Remover 1262
- Development Weights
- QuickGel LD Vis Isoenzyme Reagent (10)

Storage and Stability:

Sample as LD5 is very unstable at freezing temperatures. If testing more than 10 gels at once, place cups into both rows. 8. Repeat this until the reagent has been spread four times in each well or until the reagent relocates along the rod.

5. Open the lid of the incubation chamber. 9. Pour the contents of the vial of reagent along the anode edge of the gel. Place the gel into the preheated (45°C) Incubation Chamber.

6. When the gel has completed the process, the instrument will beep. Carefully remove the SPIFE QuickGel Holder from the stainer chamber. While holding the gel agarose side down, slide one side of the gel into the preheated chamber floor. 2. Dispense approximately 1 mL of REP Prep onto the left side of the gel and then apply fingertip pressure to the template. Remove the blotter.

NOTE:

The SD gels should be scanned for quantitative results within two hours of completion. The gel may be kept an indefinite period of time as a permanent record. A representative scan of a LD isoenzyme pattern.

The following instructions are for using the QuickGel Chamber (Cat. No. 3538T). Individual items are contained in the QuickGel LD Kit (Cat. No. 3538T). The following items are contained in the QuickGel LD Kit:

- QuickGel Chamber
- QuickGel LD Vis Isoenzyme Reagent (10)
- QuickGel Gel Block Remover 1262
- Development Weights

The following instructions are for using the QuickGel Chamber (Cat. No. 3538T). Individual items are contained in the QuickGel LD Kit (Cat. No. 3538T). The following items are contained in the QuickGel LD Kit:

- QuickGel Chamber
- QuickGel LD Vis Isoenzyme Reagent (10)
- QuickGel Gel Block Remover 1262
- Development Weights

The primary step in the Qualitative Evaluation is visual inspection. The following items are contained in the QuickGel LD Kit:

- Qualitative evaluation: The QuickGel LD Gel may be visually inspected to detect the presence of the bands.
- Quantitative evaluation: Scan the QuickGel LD Gel in QuickScan 2000. The band results may also be quantified to verify the accuracy of quantitative evaluation.

Note that the QuickGel LD Reagent is sensitive to 3 U/L. Results from sensitivity studies showed that each incubation, wash, and electrophoresis step must be completed within 10 minutes of each other. 4. Using an in situ applicator, cytology or electrophoretic Application Module from the packaging. 10. At the end of incubation, remove the gel from the incubator. Place the gel in the QuickScan 2000. If the gel is too hot, let it cool to room temperature before scanning. 12. Ensure the QuickGel LD Reagent holder from the package, if not, cut the gel from the packaging. All washes and destains are held. Hold the template on the small side of the stainer and run for 7.5 minutes. 45°C. 5. Open the lid of the incubation chamber. 9. Pour the contents of the vial of reagent along the anode edge of the gel. Place the gel into the preheated (45°C) Incubation Chamber.

2. Place the Cup Tray with samples on the SPIFE 2000. Align the sample numbers on the Tray with the numbered pins on the instrument. 3. Place the left notch of the gel so that it fits the pin of the center of the reagent relocates along the rod.

Item

QuickGel LD Vis Isoenzyme Reagent (10)
QuickGel Chamber
QuickGel Gel Block Remover 1262
Development Weights

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IV. Electrophoresis and Incubation

1. Place the Cup Tray with samples on the SPIFE 2000. Align the sample cups into both rows. (Refer to Machine Operation Manual for instructions on loading sample cups onto the SPIFE QuickGel Holder.) The cup holder must be placed in the electrophoresis chamber. The electrophoresis chamber will apply the samples, electrophorese, and beep when finished.

2. The QuickGel LD Vis Isoenzyme Reagent (10)

3. Place the left notch of the gel so that it fits the left pin of the chamber floor. Place the right notch of the gel so that it fits the right pin of the chamber floor. Use a lint free tissue to wipe around the edges of the gel backing to remove any remaining reagent. Once dried, the gel should be protected from light in the interim. Gels are not available.

4. Store the QuickGel LD Vis Isoenzyme Reagent at room temperature (20°C). Do not use after 2 years. This is a recommended pH range. The pH of each kit is maintained within this range to ensure proper absorption.

5. Place the gel on the QuickGel Blotter D. Close the chamber and place it in a laboratory incubator at 30°C for 24 hours. Do not store above 30°C or below 15°C.

6. Press the button. Powdered form will appear in gel wells and the QuickGel Blotter D becomes moist. Remove the QuickGel Blotter D. Dispose of the QuickGel Blotter D as biohazardous waste.

A representative scan of a LD isoenzyme pattern.

VI. Analysis

1. Qualitative evaluation: The QuickGel LD Gel may be visually inspected after incubation. The most anodic zone (LD1) migrates with a mobility similar to alpha 1 antitrypsin. Following electrophoresis, five zones of LD activity can be demonstrated. The most anodic zone (LD1) migrates with a mobility similar to alpha 1 antitrypsin. Following electrophoresis, five zones of LD activity can be demonstrated.

2. Quantitative evaluation: The QuickGel LD Gel can be scanned for densitometric analysis. A calibration curve is not necessary because relative intensity of the bands and may also be quantitated to verify the accuracy of quantitation. The following results were obtained:

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4. Limitations: Qualitative evaluation of LD activity is performed by Helena Laboratories. The following results were obtained:

5. Applications: A representative scan of a LD isoenzyme pattern.
using the SPIFE 2000 and the SPIFE 3000. N = 80

LD2 34.7 0.5 1.5%
LD1 34.3 0.8 2.3%

Both specimens were run 10 times on one gel with the fol-

4. LD4 elevation has not been associated with any particular pathology.
3. LD3 is elevated in pulmonary infarctions.16, 11, 20
2. LD1 is elevated and may be greater than LD2 in:
   a. Myocardial infarction1-4, 11
   b. Duchenne's muscular dystrophy presents a pattern like MI but clini-
   cally it is different.
1. Brish, L.K., CK & LD Isoenzymes A Self-Instructional Text, Am Soc of
5. US. Public Health Service. Guidelines for laboratory testing in the eval-

Further Testing Required:
2. In diagnosing myocardial infarction, CK isoenzyme studies should be
   done to rule out other causes of chest pain. Of this type of pattern and include cardiorespiratory diseases, malignan-
   cy, fracture, diseases of the central nervous system, infection/inflammation, and neoplasms. The major causes of MI
   may be acute coronary disease, advanced pericarditis, acute angina pectoris, direct damage to the heart muscle.

Between Run

% LD1 34.1 0.7 2.0
% LD2 33.4 1.0 2.9
% LD1/LD2 1.0 0.0 4.6

Control Fraction  Mean SD CV%

% LD4  5.7 0.5 9.5
% LD3  22.4 0.3  1.4%
% LD2  33.4 1.0  2.9
% LD4  6.5 0.7 10.0%

Ratio LD1/LD2  0.7 0.0  2.4%

Percentage normals: 3 = 30%, 4 = 30%, 30 = 20%, 40 = 20%

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**INTERPRETATION OF RESULTS**

1. LD2 is the LD isoenzyme present in the largest amount in normal muscle.
2. LD1 is elevated and may be greater than LD2 in:
   - Duchenne's muscular dystrophy presents a pattern like MI but clinically is not MI.
   - Hemolysis occurs in sickle cell anemia, pernicious, and megaloblastic anemias. Renal infarct may also cause abnormal LD isoenzymes. Haptoglobin studies should be performed to rule out hemolysis as a cause of elevated LD.
3. Haptoglobin studies should be performed to rule out hemolysis as a cause of elevated LD.
4. Further testing required when total LD is markedly elevated but all the isoenzymes are of normal percentages, the phenomenon is referred to as an isomorphic shift.

**REFERENCES**

1. QuickGel LD Kit Cat. No. 3338
15. Pro 77