

Cat. No. 3338, 3538T

The QuickGel LD Procedure is intended for the qualitative and quantitative analysis of the lactate dehydrogenase isoenzymes using agarose gel electrophoresis on the SPIFE 2000/3000 system or on the QuickGel Chamber.

Cat. No. 3338 – for use with SPIFE 2000/3000

Cat. No. 3538T – for use with QuickGel Chamber

SUMMARY

Lactate dehydrogenase (LD, EC 1.1.1.27) is an enzyme found in virtually all human tissues with the liver, skeletal muscle, heart and kidney having the greatest concentrations. The wide distribution of LD in body tissues limits the usefulness of total LD determinations in diagnoses. Definitive testing for the source of elevated LD activity may be accomplished with isoenzyme assessment.¹

Five isoenzymes of LD can be demonstrated in human serum. Each isoenzyme is designated by a number which is related to its electrophoretic mobility. The most anodic fraction is designated LD₁ and is found primarily in heart muscle. The most cathodic is LD₅, found primarily in liver and skeletal muscle. The others - LD₂, LD₃ and LD₄ - are found in varying degrees along with LD₁ and LD₅ in all tissues. Since LD₂ is found in highest concentration in normal human serum, the ratio LD₁/LD₂ is therefore less than one. Approximately 12-24 hours following myocardial infarction (MI), there is substantial elevation in LD₁ so that the LD₁/LD₂ ratio following MI is generally greater than 1, a phenomenon referred to as "flipped LD". Peak activity is usually reached on day 3-4 and activity may remain elevated for as long as two weeks after infarction.⁴ The LD "flip" can also be present in pernicious, hemolytic, acute sickle cell or megaloblastic anemias; renal necrosis or in cases of in-vitro or in-vivo hemolysis of any cause.⁵

An elevation of LD₅ can be seen in skeletal (muscle) injuries and degenerative diseases. It is also increased in many types of liver injuries such as cirrhosis, all types of hepatitis, and passive liver congestion.⁵

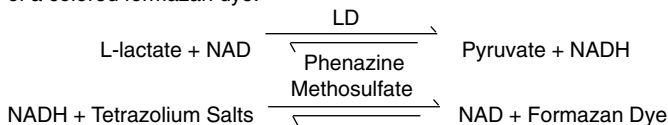
The mid-zone fractions (LD₂, LD₃, LD₄) may be elevated in cases of massive platelet destruction (pulmonary embolism) and in diseases involving the lymphatic system such as infectious mononucleosis, lymphomas and lymphocytic leukemias.⁵

The isoenzymes of LD have been determined by various methods.⁷⁻¹¹ Electrophoresis provides far more information than the other methods because it allows complete separation of all five isoenzymes with no risk of carryover. The support media used in electrophoresis includes cellulose acetate, agar, agarose and acrylamide gels.¹ The QuickGel LD Isoenzyme system is a modification of that of Preston.⁸

PRINCIPLE

The isoenzymes of LD are separated according to their electrophoretic mobility on agarose. After separation, each isoenzyme is detected colorimetrically.

Using the QuickGel LD, a tetrazolium salt is reduced with the formation of a colored formazan dye.



REAGENTS

1. QuickGel LD Gel

Ingredients: Each gel contains agarose in a sodium barbital buffer, AMPD, aspartic acid, bicine and stabilizers. Sodium azide has been added as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. The gel contains barbital which, in sufficient quantity, can be toxic. Refer to Sodium Azide Warning.

Preparation for Use: The gels are ready for use as packaged.

Storage and Stability: The gels should be stored at room temperature (15 to 30°C), in the protective packaging and are stable until the expiration date indicated on the package. **DO NOT REFRIGERATE OR FREEZE THE GELS.**

Signs of Deterioration: Any of the following conditions may indicate deterioration of the plate: (1) crystalline appearance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of

the agarose, (3) bacterial growth indicating contamination, (4) thinning of the gel blocks.

2. QuickGel LD Isoenzyme Reagent

Ingredients (after reconstitution):

NAD	10.0 mM
Lithium Lactate.....	300.0 mM
NBT	11.1 mM
PMS	0.375 mM

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.

Preparation for Use: Reconstitute each vial of reagent with 1.0 mL of SPIFE LD Diluent.

Storage and Stability: The dry reagent should be stored at 2 to 8°C and is stable until the expiration date on the vial and box. The reconstituted reagent is stable 48 hours at 2 to 8°C when stored in the dark. If exposed to the light, the color will change from yellow to green to blue. This does not affect the performance characteristics of the reagent.

Signs of Deterioration: If the unreconstituted reagent is not a uniformly pale or light yellow, dry powder, it should not be used.

3. LD Diluent

Ingredients: The diluent is an AMP, bicine, barbital, aspartate buffer with sodium azide added as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. Refer to Sodium Azide Warning.

Preparation for Use: The diluent is ready for use as packaged.

Storage and Stability: The diluent should be stored at 2 to 8°C, and is stable until the expiration date on the bottle.

4. Citric Acid Destain

Ingredients: After dissolution, the destain contains 0.3% (w/v) citric acid.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST – IRRITANT.

Preparation for Use: Pour 11 L of deionized water into the Destain vat. Add the entire package of Destain. Mix well until completely dissolved.

Storage and Stability: Store the Destain at 15 to 30°C. It is stable until the expiration date on the package.

Signs of Deterioration: Discard if solution becomes cloudy.

Sodium Azide Warning

To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions. When discarding reagents containing sodium azide, always flush sink with copious quantities of water. This will prevent the formation of metallic azides which, when highly concentrated in metal plumbing, are potentially explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.

INSTRUMENTS

A SPIFE 2000/3000 or the QuickGel Chamber must be used to electrophorese the gels. A high quality scanning densitometer such as the QuickScan 2000 (Cat. No. 1660) may be used to scan the gels.

SPECIMEN COLLECTION AND HANDLING

Specimen: Serum is the specimen of choice. Plasma from blood specimens collected in heparin or EDTA may be used. Anticoagulants containing oxalate should not be used due to the inhibition of LD by oxalate.¹¹ Plasma samples should be well centrifuged to eliminate platelets which contain LD.¹²

Interfering Substances:

- Hemolysis: Erythrocytes contain 100 to 150 times more LD than does serum. Hemolysis may contribute to error in assessment of LD_{1,2} activity.^{1,2,11}
- Uremic sera: LD activity is reduced in uremic sera due to the presence of the inhibitors, urea and oxalate, and other unidentified substances. Urea affects LD₅ more than LD₁.¹³
- Acetone and chloroform inactivate all isoenzymes of LD except LD₁.¹⁴
- For the effect of various drugs on LD activity, refer to Young et al.¹⁵

Storage and Stability: Serum should be tested as soon as possible after collection. Fresh serum is the specimen of choice because different storage conditions have varying effects on the isoenzymes.^{11,14,16,17} No one storage temperature is optimum for all the isoenzymes. When storage is required, serum samples may be stored at 15 to 30°C or at 2 to 8°C for up to 48 hours. Storage at 2 to 8°C permits simultaneous storage of serum for both CK and LD isoenzyme studies.¹¹ Do not freeze the sample as LD₅ is very unstable at freezing temperatures.¹¹

PROCEDURE

Materials Provided: The following materials are provided in the QuickGel LD Kit (Cat. No. 3338). Individual items are not available.

- QuickGel LD Gels (10)
- LD Reagent (10 x 1.0 mL)
- LD Diluent (1 x 10 mL)
- QuickGel Blotter C (10)
- Citric Acid Destain (1 pkg)
- Applicator Blade Assembly-20

Materials provided by Helena but not contained in the kit:

	Cat. No.
SPIFE 3000	1088
SPIFE 2000	1130
QuickScan 2000	1660
REP Prep	3100
QuickGel Dispo Cup Tray	3353
SPIFE QuickGel Electrodes	1111
SPIFE QuickGel Gel Holder	3358
CK/LD Control	5134
Gel Block Remover	1115
SPIFE 3000/REP 3 Reagent Spreaders	3706
SPIFE Dispo Sample Cups (Deep Well) Chamber Cover	8JP34012
SPIFE QuickGel Chamber Alignment Guide	86541003
SPIFE Applicator Blades	3450
SPIFE Reagent Spreader	3386

STEP-BY-STEP METHOD

I. Stainer Preparation

NOTE: If a SPIFE procedure requiring a stain has been run prior to running the LD gels, the stainer unit must be cleaned/washed before washing the gel.

SPIFE 3000

The SPIFE 3000 has an automatic wash cycle prompted by initiation of a test which does not use the stainer unit for staining when the previous test did use the stainer for staining. To avoid delays after electrophoresis, this wash cycle should be initiated at least seven (7) minutes prior to the end of the run. To verify the status, press the **TEST SELECT/CONTINUE** button on the stainer until the appropriate test is selected. Place an empty Gel Holder in the stainer unit. If cleaning is required, the "Wash 1" prompt will appear, followed by "Plate out, Holder in" prompts. Press "Continue" to begin the stainer wash. The cleaning process will complete automatically in about 7 minutes. The unit is then ready to process the gel after electrophoresis.

SPIFE 2000

If utilizing the unit for both stained and non-stained gels, log usage to determine when cleaning is necessary. Create a program to clean the unit as a "User Test" according to the following:

1) No Prompt			
Wash 1	1:00	REC=ON	VALVE=7
2) No Prompt			
Wash 2	1:00	REC=ON	VALVE=7
3) No Prompt			
Wash 3	1:00	REC=ON	VALVE=7
4) No Prompt			
Wash 4	1:00	REC=ON	VALVE=7
5) No Prompt			
END OF TEST			

II. Chamber Preparation

1. The SPIFE QuickGel Chamber Alignment Guide must be used to mark the location for gel placement on the chamber floor if not marked previously. It is recommended that the markings be placed directly on the copper floor under the contact sheet.
2. Remove the contact sheet and clean the chamber floor according to instructions in the Operator's Manual.
3. Place the round hole in the guide over the left chamber pin and the obround hole over the right pin.

4. Using an indelible marker, outline the rectangular open area onto the copper floor. Allow marking to dry, and apply another contact sheet.

III. Sample Preparation

1. Remove one Disposable Applicator Blade from the packaging. If testing more than 10 samples, remove two Applicator Blades from the packaging. Remove the protective guards from the blades by gently bending the protect piece back and forth until it breaks free.
2. Place the Applicator Blade into the vertical slot numbered 6 in the Applicator Assembly. If using two Applicator Blades, place them into the vertical slots numbered 6 and 12. Additional blades must be ordered (Cat. No. 3450) if testing 11 to 20 samples per gel. **Please note that the blade assembly will only fit into the slots one way; do not try to force the blade assembly into the slots.**
3. Slide the Disposable Sample Cups into the appropriately numbered top row of the Cup Tray. If testing more than 10 samples, place cups into both rows.
4. Pipette 75 to 80 µL of patient sample or control into cups 1 to 5 and 6 to 10. If testing more than 10 samples, pipette sample into cups 11 to 15 and 16 to 20. Cover the tray until ready to use.



IV. Gel Preparation

1. Carefully open one end of the pouch and remove one gel from the protective packaging. Reseal the pouch with tape to prevent drying of the gel. Remove the gel from the plastic mold and discard the mold.
2. Using a QuickGel Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
3. Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
4. Place the gel over the REP Prep inside the rectangle on the chamber floor. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side.
5. Use lint-free tissue to wipe around the edges of the gel backing to remove excess REP Prep. Make sure the gel remains in place and that no bubbles remain under the gel.
6. Clean the QuickGel Electrodes and Reagent Spreaders with deionized water before and after each use. Wipe with a lint-free tissue.
7. Place a QuickGel Electrode on the outside ledge of each gel block inside the magnetic posts. Close the chamber lid.
8. Press the **TEST SELECT/CONTINUE** button located on the Electrophoresis and Stainer sides of the instrument until the appropriate test name appears on the displays.

V. Preparation of Reagent

1. Reconstitute one vial of the LD Isoenzyme Reagent with 1.0 mL LD Isoenzyme Diluent.
2. Mix well by inversion.
3. If using the SPIFE 3000, place the reconstituted vial of reagent in the center hole of the reagent bar, ensuring that the vial is pushed down as far as it can go. Close the chamber lid.

VI. Electrophoresis/Visualization

Using the instructions provided in the appropriate Operator's Manual, set up the parameters as follows for the SPIFE 2000 or the SPIFE 3000.

NOTE: A "Dry 1" time of 13 minutes is recommended. However, due to variations in environmental conditions, the following range is acceptable.

* Dry 1 = 10 to 17 minutes.

A. SPIFE 3000

Electrophoresis Unit

1) No Prompt				
Load Sample 1	00:10	20°C	SPD6	
2) No Prompt				
Apply Sample 1	1:00	20°C	SPD6	LOC 1
3) No Prompt				
Electrophoresis 1	4:00	12°C	550 V	70 mA
4) Remove Gel Blocks (Continue)				
Apply Reagent 1		45°C	4 Cycles	
5) To Continue (Continue)				
Incubate 1	20:00	45°C		
6) No Prompt				
END OF TEST				

Stainer Unit

- | | | | |
|--------------|--------|---------|---------|
| 1) No Prompt | | | |
| Destain 1 | 10:00 | REC=REV | VALVE=2 |
| 2) No Prompt | | | |
| Wash 1 | 5:00 | REC=REV | VALVE=7 |
| 3) No Prompt | | | |
| Dry 1 | *13:00 | 70°C | |
| 4) No Prompt | | | |
| END OF TEST | | | |

1. Place the Cup Tray with samples on the SPIFE 3000. Align the holes in the tray with the pins on the instrument.
2. With **QG-LD** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** again to begin. The SPIFE 3000 will apply the samples, electrophoresis, and beep when finished.
3. Open the lid, remove the QuickGel Electrodes and dispose of blades as biohazardous waste.
4. With the gel still in the chamber, use a Gel Block Remover to completely remove and discard the two gel blocks.
5. Use a lint-free tissue to wipe around the edges of the gel.
6. Place a Reagent Spreader Rod (glass rod) across each end of the gel inside the magnetic posts. Close the chamber lid and press **TEST SELECT/CONTINUE** button to spread the reagent.
7. After the reagent is spread, the instrument will beep. Open the chamber lid and insert a Chamber Cover in the grooves of the chamber. Close the chamber lid.

B. SPIFE 2000

Electrophoresis Unit

- | | | | |
|------------------------------------|-------|------|--------|
| 1) No Prompt | | | |
| Load Sample 1 | 00:10 | 20°C | SPD.=6 |
| 2) No Prompt | | | |
| Apply Sample 1 | 1:00 | 20°C | SPD.=6 |
| 3) No Prompt | | | |
| Electrophoresis 1 | 4:00 | 12°C | 550 V |
| 4) Apply/Spread Reagent (Continue) | | | |
| Incubate 1 | 20:00 | 45°C | |
| 5) No Prompt | | | |
| END OF TEST | | | |

Stainer Unit

- | | | | |
|--------------|--------|--------|---------|
| 1) No Prompt | | | |
| Destain 1 | 10:00 | CIR=ON | VALVE=2 |
| 2) No Prompt | | | |
| Wash 1 | 5:00 | CIR=ON | VALVE=7 |
| 3) No Prompt | | | |
| Dry 1 | *13:00 | 70°C | |
| 4) No Prompt | | | |
| END OF TEST | | | |

1. Place the Cup Tray with samples on the SPIFE 2000. Align the holes in the tray with the pins on the instrument. Close the chamber lid.
2. With **LDH** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The SPIFE 2000 will apply the samples, electrophoresis, and beep when finished.
3. Open the chamber lid, remove the electrodes and dispose of blades as biohazardous waste.
4. With the gel still in the chamber, use a Gel Block Remover to completely remove and discard the two gel blocks.
5. Use a lint-free tissue to wipe around the edges of the gel.
6. Pour the contents of the SPIFE LD Reagent vial across the cathode end of the gel. Use the Reagent Spreader to work the reagent into the gel. Set the ends of the Reagent Spreader on the inner ledge of the electrophoresis chamber with the rod laying on the gel inside the posts.
7. Slowly slide the spreader across the gel. Tilt the spreader so the reagent relocates along the rod.
8. Repeat this until the reagent has been spread four times in each direction. On the last spread across the gel, continue to drag the rod off the end of the gel to dispose of excess reagent.
9. Replace carbon electrodes on each side of the gel inside the magnetic posts. Insert a Chamber Cover in the grooves of the chamber. Close the chamber lid.

VII. Incubation

1. Press the **TEST SELECT/CONTINUE** button to start the incubation timer.
2. Once incubation is complete, the instrument will beep. Open the chamber lid. Remove the Chamber Cover and gel from the chamber.
3. Remove the SPIFE QuickGel Holder from the stainer chamber. While holding the gel agarose side down, slide one side of the gel backing under one of the metal bars. Bend the gel backing so that the gel is bowed, and slip the other side under the other metal bar. The two small notches in the backing must fit over the small pins to secure the gel to the holder.
4. Place the SPIFE QuickGel Holder with the attached gel facing backwards into the stainer chamber.
5. With the appropriate test name displayed, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The instrument will destain, wash and dry the gel.
6. When the gel has completed the process, the instrument will beep. Carefully remove the SPIFE QuickGel Holder from the stainer because the metal piece on the holder will be hot.

PROCEDURE FOR QuickGel® CHAMBER

The following instructions are for using the QuickGel Chamber (Cat. No. 1284) for electrophoresis.

Materials Provided: The following materials needed for the procedure are contained in the QuickGel LD Kit (Cat. No. 3538T). Individual items are not available.

- QuickGel LD Gels (10)
- QuickGel LD Vis Isoenzyme Reagent (10)
- QuickGel LD Diluent (1)
- QuickGel Blotter C (10)
- QuickGel Blotter D (10)
- Citric Acid Destain (1 pkg)
- QuickGel Templates (10)
- QuickGel Blotter A (10)

Materials provided but not contained in the kit:

Item	Cat. No.
QuickScan 2000	1660
QuickGel Chamber	1284
Development Weight	5014
Incubation Chamber	4062
REP Prep	3100
QuickGel Gel Block Remover	1262
CK/LD Isoenzyme Control	5134
QuickGel Accessory Kit	3426
Staining Dish	4061

Materials needed but not provided:

- Incubator capable of maintaining 45°C
- 5 mL serological pipette
- Power Supply capable of providing at least 600 Volts.

STEP BY STEP METHOD

I. Chamber Preparation

1. The QuickGel Chamber must be plugged into a power supply. Set a timer for *2:45 minutes and the power at 600 Volts. *An electrophoresis time of 2:30 to 3:00 minutes is acceptable.
2. Snap the Electrophoresis Lid into place on the chamber.
3. Ensure that the chamber floor is cool (room temperature) before starting the test.

II. Preparation of the Incubation Chamber

1. Place a QuickGel Blotter D in the bottom of the incubation chamber.
2. Wet the blotter completely with water and then pour off excess.
3. Close the chamber and place it in a laboratory incubator at 45°C.
4. Allow the incubation chamber and blotter to equilibrate to 45°C while performing the electrophoresis steps.
5. If more than one gel will be put in the incubator, it is recommended that each incubation chamber be placed between preheated Development Weights.
6. Reconstitute the QuickGel LD Reagent with 1.0 mL LD Diluent. Mix well by inversion.

III. Sample Template Application

1. Carefully cut open one end of the gel pouch. Remove one gel from the protective packaging. Fold and tape the open end of the pouch to prevent drying of the remaining gel. Remove the gel to be used from the plastic mold and discard the mold.
2. Dispense approximately 1 mL of REP Prep onto the left side of the electrophoresis chamber.
3. Place the left notch of the gel so that it fits the left pin of the chamber floor and gently roll the gel to the right side fitting the right notch to the right pin of the chamber floor. Use a lint free tissue to wipe around the edges of the gel backing to remove any excess REP Prep. Make sure that no bubbles remain under the gel.
4. Using a QuickGel Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
5. Remove one QuickGel Template from the package if only one row of samples is tested. Remove two templates if 11 to 20 samples are tested. Hold the template so that the small hole in the corner is toward the front right side of the chamber.
6. Carefully place the template on the gel aligning the template slits with the marks on each side of the gel backing. The center hole in the template should align with the indentation in the center of the gel.
7. Apply slight fingertip pressure to the template making sure there are no bubbles under it. **NOTE:** If wearing rubber gloves to perform this step, place a QuickGel Blotter A over the template and then apply fingertip pressure to the template. Remove the blotter. Powder from the gloves can produce gel artifacts.
8. Apply 2 μ L of the appropriate sample to the template slits. After the last sample application, wait 3 minutes to allow time for the proper absorption.
9. Use the QuickGel Blotter A to gently blot the excess sample from the template. Carefully remove the blotter and template and dispose of them as biohazardous waste.
10. Close the lid, press the power switch to turn on the chamber and start the power supply.

IV. Electrophoresis and Incubation

1. Electrophorese the gel for *2:45 minutes at 600 Volts.
2. Turn off the Power Supply and the QuickGel Chamber.
3. Open the lid. Using the QuickGel Gel Block Remover, remove the two gel blocks from the gel. Again, use a lint free tissue to wipe around the edges of the gel backing to remove any excess moisture.
4. Remove the gel from the chamber and place it, agarose side up, on a clean nonporous surface with the cathode edge (top of gel) away from you.
5. Pour the contents of the vial of reagent along the cathode edge of the gel.
6. Lay a 5 mL serological pipette lengthwise along the cathode edge of the gel. Gently spread the reagent by slowly pulling the pipette across the agarose to the anode edge, being careful not to roll the reagent off the gel. Pause for a couple of seconds. Then pull the pipette across the gel from anode to the cathode edge in the same manner. Pause for a couple of seconds. Then pull the pipette across the gel from cathode to anode and roll the excess off the gel. Use a lint free tissue to wipe around the edges of the gel.
7. Place the gel into the preheated (45°C) Incubation Chamber.
8. Place the Incubation Chamber in a laboratory incubator at 45°C for 20 minutes.
9. Two Staining Dishes are needed; one for the Destain Solution and one for the water wash. Pour a sufficient amount of Destain into one dish to cover the gel. Pour the same amount of water into the other dish.
10. At the end of incubation, remove the gel from the incubator. Place the gel horizontally into the destain solution and destain using a gentle alternately rocking and swirling technique. Allow the gel to remain in the destain solution for 10 minutes. Remove the gel and tap it to get rid of excess destain.
11. Place the gel horizontally into the container of water for 5 minutes. Use the same technique as in Step 10 to wash the gel. Tap the gel to remove the excess water.
12. Ensure the chamber floor is clean, and place the Drying Lid on the chamber. Replace the gel onto the chamber floor. Close the Drying Lid, turn the chamber on and dry the gel for 20 minutes or until dry. Remove the gel when drying is completed, and turn off the chamber.

Evaluation of LD Isoenzyme Bands

1. Qualitative evaluation: The QuickGel LD Gel may be visually inspected for the presence of the bands.
2. Quantitative evaluation: Scan the QuickGel LD Gel in QuickScan 2000 using the Acid Violet Filter and slit 5.

Stability of End Product

The LD gels should be scanned for quantitative results within two hours after drying. The gel should be protected from light in the interim. Gels may be kept an indefinite period of time as a permanent record.

Calibration

A calibration curve is not necessary because relative intensity of the bands is the only parameter determined.

Quality Control

The CK/LD Isoenzyme Control (Cat. No. 5134) can be used to verify all phases of the procedure and should be used on each plate run. The control should be used as a marker for proper location of the isoenzyme bands and may also be quantitated to verify the accuracy of quantitations. Refer to the package insert provided with the control for assay values. Additional controls may be required for federal, state or local regulations.

REFERENCE VALUES

Reference range studies including fifty (50) men and women were performed by Helena Laboratories. The following results were obtained:

LD ₁	=	17.7 - 31.5
LD ₂	=	28.0 - 35.7
LD ₃	=	20.8 - 26.8
LD ₄	=	6.4 - 12.7
LD ₅	=	4.5 - 16.0
LD ₁ /LD ₂	=	0.5 - 1.0

These values should only serve as guidelines. Each laboratory should establish its own expected value range with this procedure.

RESULTS

Following electrophoresis, five zones of LD activity can be demonstrated. The most anodic zone (LD₁) migrates with a mobility similar to alpha₂ globulin. The most cathodic zone (LD₅) travels with the gamma globulin and the remaining three zones have intermediate mobilities. The LD activity in normal serum reflects the break-down of numerous cells and all 5 components can be seen. LD₂ predominates, followed by LD₁ and LD₃. LD₄ and LD₅ occur only in minor amounts.



Figure 1: QuickGel LD Gel showing the relative position of the LD Isoenzyme bands.

Calculation of the Unknown

The Helena QuickScan 2000 will automatically calculate and print the relative percent and the absolute values for each band. Refer to the Operator's Manual provided with the densitometer.

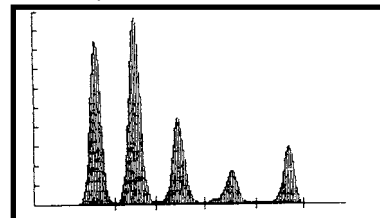


Figure 2: A representative scan of a LD isoenzyme pattern.

LIMITATIONS

The QuickGel LD Isoenzyme Reagent, when used on the SPIFE, is linear to a total LD of 1000 U/L. When used on the QuickGel Chamber, the linearity is to at least 500 U/L. Samples with values greater than this should be diluted with deionized water. Results from sensitivity studies showed that the QuickGel LD Reagent is sensitive to 3 U/L.

NOTE: The QuickGel LD method is not designed to identify tumor markers

Interfering Factors:

Refer to **SPECIMEN COLLECTION AND HANDLING.**

Further Testing Required:

1. Total LD activity may be determined. Conflicting reports exist about the true value of total serum enzyme levels as compared to the severity of a disease.^{1, 4, 22}
2. In diagnosing myocardial infarction, CK isoenzyme studies should be performed.^{1, 4}
3. Haptoglobin studies should be performed to rule out hemolysis as a cause of elevated LD₁ and LD₂.

INTERPRETATION OF RESULTS

1. LD₂ is the LD isoenzyme present in the largest amount in normal serum.^{1-4, 11}
2. LD₁ is elevated and may be greater than LD₂ in:
 - a. Myocardial infarction^{1-4, 11}
 - b. Duchenne's muscular dystrophy presents a pattern like MI but clinical symptoms help in easily differentiating the two diseases.¹⁸⁻¹⁹
 - c. Hemolysis (including Hemolytic anemias) should be strongly considered whenever total serum LD reaches levels greater than 5 times normal and the isoenzymes show an increased LD₁ and LD₂. Total LD is much higher in hemolytic anemia than in MI unless MI is accompanied by severe shock. Pernicious anemia (PA) in relapse gives an LD pattern like hemolysis. Some of the highest total serum LD values are found in PA.^{2, 14}
 - d. Renal infarct^{2, 11}
3. LD₃ is elevated in pulmonary infarctions.^{16, 11, 20}
4. LD₄ elevation has not been associated with any particular pathology.
5. LD₅ is elevated in hepatic and muscular damage and diseases of the skin.¹
6. Isomorphic patterns
When total LD is markedly elevated but all the isoenzymes are of normal percentages, the phenomenon is referred to as an isomorphic pattern. Widely divergent groups of clinical diagnoses have shown this type of pattern and include cardiorespiratory diseases, malignancy, fracture, diseases of the central nervous system, infection/inflammation, hepatic cirrhosis and/or alcoholism, trauma without fracture, infectious mononucleosis, hypothyroidism, uremia, necrosis, pseudo-mononucleosis, viremia and intestinal obstruction. (See LIMITATIONS Note)
7. CK and LD values following open heart surgery:
CK and LD isoenzymes are less specific following open heart surgery than they are in most diagnostic situations. The CK-MB will be elevated due to myocardial damage resulting from the operative procedure as well as trauma to the heart from manipulation and cannulation. The LD₁/LD₂ ratio may be elevated secondary to hemolysis from extra corporeal circulation.

PERFORMANCE CHARACTERISTICS

SPIFE

PRECISION

A normal patient sample and an abnormal control were tested on two lots of gel. The gels were run on both the SPIFE 2000 and SPIFE 3000.

Within Run: Both specimens were run 10 times on one gel with the following results. N = 10.

Normal Serum

Fraction	Mean	SD	CV
LD ₁	23.8	0.3	1.3%
LD ₂	35.0	0.3	0.8%
LD ₃	22.5	0.2	0.8%
LD ₄	8.8	0.2	2.5%
LD ₅	9.9	0.4	3.5%
Ratio LD ₁ /LD ₂	0.7	0.0	1.7%

Control

Fraction	Mean	SD	CV
LD ₁	34.3	0.8	2.3%
LD ₂	34.7	0.5	1.5%
LD ₃	17.6	0.5	2.9%
LD ₄	6.7	0.5	7.0%
LD ₅	6.6	0.5	6.9%
Ratio LD ₁ /LD ₂	1.0	0.0	2.4%

Between Run: Both specimens were analyzed in replicate on eight gels using the SPIFE 2000 and the SPIFE 3000. N = 80

Normal Serum

Fraction	Mean	SD	CV
LD ₁	23.7	0.5	2.2%
LD ₂	35.3	0.4	1.2%
LD ₃	22.4	0.3	1.4%
LD ₄	8.9	0.3	3.7%
LD ₅	9.7	0.5	5.2%
Ratio LD ₁ /LD ₂	0.7	0.0	2.4%

Control

Fraction	Mean	SD	CV
LD ₁	34.9	1.0	2.8%
LD ₂	35.1	0.6	1.8%
LD ₃	16.7	0.9	5.2%
LD ₄	6.5	0.7	10.0%
LD ₅	6.8	0.8	12.1%
Ratio LD ₁ /LD ₂	1.0	0.0	2.8%

CORRELATION

Fifty normal and fifty abnormal patient specimens plus a control were analyzed using both the SPIFE 2000 and SPIFE 3000. The SPIFE LD method was used as the reference method.

N	=	101
Slope	=	0.958
Intercept	=	0.8449
R	=	0.9953
Y	=	0.958X + 0.8449
X	=	SPIFE LD
Y	=	QuickGel LD on SPIFE

LINEARITY

QuickGel LD showed linearity up to a total LD of 1000 U/L.

QuickGel Chamber

PRECISION

Within Run studies were run using one control run in replicate on one gel.

Control	Fraction	Mean	SD	CV%
N = 20				
	% LD ₁	34.1	0.7	2.0
	% LD ₂	32.9	0.6	1.9
	% LD ₃	16.0	0.9	5.7
	% LD ₄	5.7	0.5	9.5
	% LD ₅	11.3	0.9	7.8
	% LD ₁ /LD ₂	1.0	0.0	4.6

Between Run studies were done using one control run in replicate on eight gels.

Control	Fraction	Mean	SD	CV%
N = 160				
	% LD ₁	33.9	1.2	3.6
	% LD ₂	33.4	1.0	2.9
	% LD ₃	16.4	1.1	6.6
	% LD ₄	5.4	0.9	16.0
	% LD ₅	10.9	1.1	10.2
	% LD ₁ /LD ₂	1.0	0.0	4.2

CORRELATION STUDIES

A total of 57 (17 normal and 40 abnormal) patient specimens were tested on the QuickGel LD method using the SPIFE and the QuickGel Chamber.

N	=	57	Y =	1.0081X - 0.1475
Slope	=	1.0081	Y =	QuickGel LD on SPIFE
Intercept	=	-0.1475	Y =	QuickGel LD on QuickGel Chamber
R	=	0.997		

LINEARITY

QuickGel LD showed linearity up to a total LD of 500 U/L.

SENSITIVITY

Results from validation studies show that the system is sensitive to 3 U/L.

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QuickGel LD System

QuickGel LD Kit

Cat. No. 3338

- QuickGel LD Gels (10)
- LD Reagent (10 x 1.0 mL)
- LD Diluent (1 x 10 mL)
- QuickGel Blotter C (10)
- Citric Acid Destain (1 pkg)
- Applicator Blade Assembly-20

Other Supplies and Equipment

The following items, needed for performance of the QuickGel LD Procedure, must be ordered individually.

	Cat. No.
SPIFE 3000	1088
SPIFE 2000	1130
QuickScan 2000	1660
REP Prep	3100
QuickGel Dispo Cup Tray	3353
SPIFE QuickGel Electrodes	1111
SPIFE QuickGel Gel Holder	3358
CK/LD Control	5134
Gel Block Remover	1115
SPIFE 3000/REP 3 Reagent Spreaders	3706
SPIFE Dispo Sample Cups (Deep Well)	3360
Chamber Cover	8JP34012
SPIFE QuickGel Chamber Alignment Guide	86541003
SPIFE Applicator Blades	3450
SPIFE Reagent Spreader	3386

QuickGel LD Kit

Cat. No. 3538T

- QuickGel LD Gels (10)
- QuickGel LD Isoenzyme Reagent (10)
- QuickGel LD Diluent (1)
- QuickGel Blotter C (10)
- QuickGel Blotter D (10)
- Citric Acid Destain (1 pkg)
- QuickGel Templates (10)
- QuickGel Blotter A (10)

Other Supplies and Equipment

Item	Cat. No.
QuickScan 2000	1660
QuickGel Chamber	1284
Development Weights	5014
Incubation Chamber	4062
REP Prep	3100
QuickGel Gel Block Remover	1262
CK/LD Isoenzyme Control	5134
QuickGel Accessory Kit	3426
Staining Dish	4061

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