The SPIFE Touch LD Isoenzyme procedure is intended for the qualitative and quantitative analysis of the lactate dehydrogenase isoenzymes in serum or plasma by agarose electrophoresis using the SPIFE Touch system.

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 concentration. The wide distribution of LD in body tissues limits the usefulness of total LD determinations in diagnosis. Testing for the source of elevated LD activity may be indicated with enzyme assessment.

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 cathodal LD3 is found primarily in liver and skeletal muscle. The other LD isoenzymes, LD4 and LD5, are found in varying degrees along with LD1 and LD2 in all tissues.1 Since LD1 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/LD ratio following MI will approach or even exceed 1, a phenomenon referred to as "fliped LD." Peak activity is usually reached on day 3-4 and activity may remain elevated for as long as two weeks after infarction. The LD "flip" may also be present in pernicious, hemolytic, acute sickle cell or megoblastic anemias; renal necrosis or in cases of in-vitro or in-vivo hemolysis of any cause.2

An elevation of LD 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. The mid-zone fractions (LD4, LD5) may be elevated in cases of massive platelet destruction (purpura fulminans) and in diseases involving the lymphatic system such as infectious mononucleosis, lymphomas and lymphocytic leukemias. The isoenzymes of LD have been determined by various methods.3-5 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.6 The SPIFE LD system is a modification of that of Preston and Grant.3

PRINCIPLE
The isoenzymes of LD are separated according to their electrophoretic mobility on agarose. After separation, each isoenzyme is detected colorimetrically. Using the SPIFE LD Isoenzyme System, a tetrazolium salt is reduced with the formation of a colored final product.

Lactate + NADH + Tetrazolium Salts

Lactate

LD

NADH + Pyronine + NAD

Tetrazolium Salts

Pyronine

NAD

Methysulphate

Farnby

Reagents

1. SPIFE LD Isoenzyme Gel
Ingredients: Each gel contains agaroose in a sodium barth buffer, AMPD, ascorbic acid, biocry and stabilizers. Sodium azide has been added as a preservative.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. The gel can be scanned on a densitometer such as the Quick Scan Touch/2000 (Cat. No. 1690/1660). Refer to the appropriate Operator’s Manual for detailed instructions.

2. LD Isoenzyme Reagent
Ingredients: NAD+ to Sodium Azide Warning. The LD Isoenzyme Reagent is intended for use after each reagent is dispensed to the gel.

Instruments: A SPIFE Touch must be used to electrophorese the gel. The gel can be scanned on a densitometer such as the Quick Scan Touch/2000 (Cat. No. 1690/1660). Refer to the appropriate Operator’s Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING
A SPIFE Touch is the specimen of activity, refer to Young et al.7 for more details. For the effect of various drugs on LD activity, refer to Young et al.7

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.8-15 No one storage temperature is optimal for all isoenzymes. However, some general guidelines are summarized as follows:

1. Hemolysis: Hemolysates contain about 100-150 times more LD than serum does. Hemolysis may contribute to error in assessment of LD activity.7-9

2. Uremic sera: LD activity is reduced in uremic sera due to the presence of the inhibitors, urea, oxalate and other unidentified substances. Urea affects LD activity similarly to hemolysis.

3. Acetone and chloroform inactivate all isoenzymes of LD except LD1.

4. For the effect of various drugs on LD activity, refer to Young et al.7

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3. Acetone and chloroform inactivate all isoenzymes of LD except LD1.
STEP-BY-STEP METHOD

NOTE: If the staining chamber was last used to stain a gel, the SPIFE Touch software has an automatic wash cycle prompted by the initiation of the SPIFE Touch LD Isoenzyme test. To verify the status of the stainer chamber, use the arrows to check parameters, select test and press SETUP. The instrument will wash the chamber.

I. Preparation of Staining Equipment
1. Reconstitute two vials of the LD Isoenzyme Reagent with 1.0 mL LD Isoenzyme Diluent each.
2. Mix well by inversion.

II. Sample Preparation
1. If testing 41-60 samples, remove three disposable Applicator Blades from the packaging. If testing fewer samples, remove the appropriate number of Applicator Blades from the packaging.
2. Place the three Applicator Blades into the vertical slots in the Applicator Assembly identified as 2, 3, and 6. If using fewer Applicator Blades, place them into any of the three slots noted above.

NOTE: The blade assembly will only fit on the slots one way; do not force the blade assembly into the slots.

3. Place an Applicator Blade Weight on top of each blade assembly. When the cleaning process will complete in approximately 7 minutes. To avoid delays after incubation, this wash cycle should be initiated at least 7 minutes prior to the end of the run.

4. Slide three Disposable Cup strips into rows 1, 3 and 5 of the cup tray.
5. Place an Applicator Blade Weight on top of each blade assembly. When the cleaning process will complete automatically in about 7 minutes. To avoid delays after incubation, this wash cycle should be initiated at least 7 minutes prior to the end of the run.

III. Sample Application/Electrophoresis
1. At the end of the incubation, remove the gel from the chamber and place it on a blotter, **dorso side up**. Using a blade or straight edge, completely remove excess REP Prep. Make sure no bubbles remain under the gel.
2. Place a carbon electrode on the outer edge of each gel block on the outside of the magnetic posts. Depending on the contact between the electrode and the gel block may cause skewed patterns. Close the chamber lid.
3. Open the Chamber Cover before placing the gel onto the electrodes. **Dorso side up**.
4. Place the left edge of the gel over the REP Prep aligning the right hole on the left pin of the chamber. Gently lay the gel down on the REP Prep, starting from the left and ending on the right side, filling the oblong hole over the right pin. Use lint-free tissue to wipe around the edges of the gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure no bubbles remain under the gel.
5. Close and wipe the electrodes with lint-free tissue. Do the same for the Reagent Spreaders (glass rods).
6. Place the Cup Tray with samples on the SPIFE Touch. Align the holes in the Cup Tray with the oblong holes of the tabs and the magnetically post.
7. Place a Reagent Spreader (glass rod) on each inner gel block, inside the magnetic posts.
8. Use the arrows under SEPARATOR UNIT to select the appropriate test. To check parameters, select test and press SETUP. The instrument will wash the chamber.

IV. Evaluation of the LD Isoenzyme Bands
1. Qualitative evaluation: The SPIFE LD Isoenzyme Gel may be visually inspected for the bands.
2. Quantitative evaluation: Scan the SPIFE LD Isoenzyme Gel in the Quick Scan Touch/2000 on the Acid Violet setting using slit 5.

REFERENCE VALUES

Reference range studies including fifty-three healthy men and women between the ages of 20 and 60 years were performed by Helena Laboratories. The following results were obtained:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD, 1</td>
<td>17.4 - 30.6</td>
</tr>
<tr>
<td>LD, 2</td>
<td>20.4 - 28.7</td>
</tr>
<tr>
<td>LD, 5</td>
<td>5.1 - 10.9</td>
</tr>
<tr>
<td>LD, 4</td>
<td>3.6 - 15.4</td>
</tr>
<tr>
<td>LD, 3</td>
<td>0.5 - 0.9</td>
</tr>
</tbody>
</table>

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 (LD 4) migrates with a mobility similar to alpha globulin. The most cathodic zone (LD 2) travels with the gamma globulin and the remaining three zones have intermediate mobilities. The LD activity in normal serum reflects the breakdown of numerous cells and all five components can be seen. LD, predominates, followed by LD, is present in moderate amounts while LD, and LD, usually occur only in minor amounts.

INTERPRETATION OF RESULTS

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STAINER UNIT

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.

INTERFERING FACTORS

1. Myocardial infarction
2. Duchenne’s muscular dystrophy
3. Hemolytic anemia
4. Myeloma
5. Renal infarction
6. Liver disease

LIMITATIONS

1. The SPIFE LD Isoenzyme Reagent is linear to a total LD of 1000 U/L. Samples with values greater than this should be diluted with distilled water. Results from sensitivity studies showed that the SPIFE LD Reagent is sensitive to 3 U/L.

Figure 2: A representative scan of a SPIFE LD pattern.
The following materials are provided in the SPIFE LD Isoenzyme Kits. Individual items are not available separately.

**Materials Provided:**

- Large Sample Cup Board: 3032
- Separator Unit
- Sample Holders: 3030
- Chamber Covers: 3066, 3031
- Reagent Bar: 3036
- Flex Cup Holder: 3035

**STEP-BY-STEP METHOD**

**I. Preparation of Isoenzyme Reagent**

1. Reconstitute two vials of the LD Isoenzyme Reagent with 1.0 mL LD Isoenzyme Diluent each.
2. Mix well by inversion.

**II. Sample Preparation**

1. If testing 41-60 samples, remove three Applicator Blades from the package. If testing fewer samples, remove the appropriate number of Applicator Blades from the packaging.
2. Place the three Applicator Blades into the vertical slots in the Applicator Assembly identified as 2, 9 and 16. If using fewer Applicator Blades, place them into any of the three slots noted above.

**III. Gel Preparation**

1. Place the gel from the protective packaging and discard overlay.
2. Remove the REP Blotter C on the gel with the longer end parallel with the gel blocks. Gently blot the entire surface of the gel using light fingertip pressure on the blotter and remove the blotter.
3. Dispense approximately 2.0 mL of REP Prep onto the left side of the electrophoresis chamber.

**IV. Sample Application/Electrophoresis**

Using the instructions provided in the Operator’s Manual, set up the parameters as follows for the SPIFE Touch:

<table>
<thead>
<tr>
<th>Separate Unit</th>
<th>Load Sample</th>
<th>Prompt</th>
<th>None</th>
<th>Time: 0:32</th>
<th>Temperature: 20°C</th>
<th>Speed: 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load Sample 1</td>
<td>Prompt</td>
<td>None</td>
<td>Time: 0:32</td>
<td>Temperature: 20°C</td>
<td>Speed: 6</td>
<td></td>
</tr>
<tr>
<td>Load Sample 3</td>
<td>Prompt</td>
<td>None</td>
<td>Time: 0:32</td>
<td>Temperature: 20°C</td>
<td>Speed: 6</td>
<td></td>
</tr>
</tbody>
</table>

**V. Washing**

1. At the end of the incubation, remove the gel from the chamber and place it on a blotter. Use a blade or straight edge, completely remove excess stain and discard the two gel blocks from the gel. The gel blocks interfere with washing. Rinse the Chamber Cover before use.
2. Place the gel Holder with the attached gel facing backwards into the stainer chamber.
3. Use the arrows under SEPARATOR UNIT to select the appropriate test. Press START and choose an operation to proceed. The instrument will wash and dry the gel.
4. When the gel has completed the process, the instrument will beep. Remove the Gel Holder from the stainer and you can scan the bands.

**VI. Evaluation of the LD Isoenzyme Bands**

1. Qualitative evaluation: The SPIFE LD Isoenzyme Gel may be visually inspected for the bands.
2. Quantitative evaluation: Scan the SPIFE LD Isoenzyme Gel in the Quick Scan Touch/2000 on the Acid Violet setting using slit 5.

**Figure 2:** A representative scan of a SPIFE LD pattern.

**LIMITATIONS**

The SPIFE LD Isoenzyme Reagent is linear to a total LD of 1000 U/L. Samples with values greater than this should be diluted with dextrose water. Results from sensitivity studies showed that the SPIFE LD Reagent is sensitive to 3 U/L.

**INTERPRETATION OF RESULTS**

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.

**Further Testing Required:**

1. Myocardial infarction
2. Duchenne’s muscular dystrophy presents a pattern like MI but clinical symptoms help to easily differentiate these two diseases.

**REFERENCES**

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

The SPIFE LD Isoenzyme Bands usually include cardiorespiratory diseases, malignancy, fracture, diseases of the central nervous system, infection/inflammation, hepatic or orthosis and alcoholism, trauma without fracture, infectious mononucleosis, hypothyroidism, uremia,
The SPIFE Touch LD Isoenzyme procedure is intended for the qualitative and quantitative analysis of the lactate dehydrogenase isoenzymes in serum or plasma by agarose electrophoresis using the SPIFE Touch system.

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 diagnosis. For testing of the source of elevated LD activity may be indicated with isoenzyme assessment.

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 LD⁵, and is found primarily in liver and heart muscle. The others - LD⁴, LD³, LD², LD¹, are in most diagnostic situations. The CK-MB will be elevated due to myocardial infarction (MI), and therefore less than one. Approximately 12-24 hours following myocardial infarction (MI), there is substantial elevation in LD⁵. Peak activity usually is reached on day 3 and activity may remain elevated for as long as two weeks after infarction. The LD⁵ may also be present in perimycotic, hemolytic, acute sickle cell or megatoblastic anemias; renal necrosis or in cases of in vitro or in vivo hemolysis of any cause.

An elevation of LD可用于 see 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.

The mid-zone fractions (LD⁴, LD³, LD²) may be elevated in cases of massive platelet destruction (pulmonary embolism) and in diseases involving the lymphatic system such as infectious mononucleosis, lymphomas and lymphoid leukemias. The isoenzymes of LD⁴ have been determined by various methods [1]. 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. The SPIFE LD system is a modification of that of Preston.

PRINCIPLE
The isoenzymes of LD are separated according to their electrophoretic mobility on agarose. On agarose, each isoenzyme is detected colorimetrically. Using the SPIFE Touch LD Isoenzyme System, a tetrazolium salt is reduced with the formation of a colored formazan dye.

SPECIMEN COLLECTION AND HANDLING
L-lactate + NAD

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

Storage and Stability: When stored at 2-6°C, the reagents are stable until the expiration date indicated on the vial. The expiration date on the vial is 1 year after the date of manufacture.

Signs of Deterioration: Discard the vial if it shows signs of bacterial growth.

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

Storage and Stability: Store the vial at 2-6°C. It is stable until the expiration date indicated on the vial. The expiration date on the vial is 1 year after the date of manufacture.

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 acids. When discarding reagents containing sodium azide, always flush with sink water and dispose of azide solutions as a hazardous waste.

To prevent the formation of metal azides which, when highly concentrated in metal plumbing, are potentially explosive. In addition, discard solutions generating toxic gases or fumes.

For IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. Refer to Sodium Azide Warning.

Inorganic pyrophosphatase
Phenazine methosulfate

Preparation for Use: For Preparations B–D, reconstitute each vial of reagent with 1.0 mL of LD Isoenzyme Diluent.

Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

Interfering Substances:

Hemolysis: When hemolysis is present, the assay results should not be used. Hemolysis may contribute to error in assay results.

Reagents:

Preparation for Use: For Preparations B–D, reconstitute each vial of reagent with 1.0 mL of LD Isoenzyme Diluent.

Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

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Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

Interfering Substances:

Hemolysis: When hemolysis is present, the assay results should not be used. Hemolysis may contribute to error in assay results.

Reagents:

Preparation for Use: For Preparations B–D, reconstitute each vial of reagent with 1.1 mL of LD Isoenzyme Diluent.

Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

Interfering Substances:

Hemolysis: When hemolysis is present, the assay results should not be used. Hemolysis may contribute to error in assay results.

Reagents:

Preparation for Use: For Preparations B–D, reconstitute each vial of reagent with 0.375 mL of LD Isoenzyme Diluent.

Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

Interfering Substances:

Hemolysis: When hemolysis is present, the assay results should not be used. Hemolysis may contribute to error in assay results.

Reagents:

Preparation for Use: For Preparations B–D, reconstitute each vial of reagent with 0.1 mL of LD Isoenzyme Diluent.

Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

Interfering Substances:

Hemolysis: When hemolysis is present, the assay results should not be used. Hemolysis may contribute to error in assay results.

Reagents:

Inorganic pyrophosphatase
Phenazine methosulfate

Preparation for Use: For Preparations B–D, reconstitute each vial of reagent with 0.037 mL of LD Isoenzyme Diluent.

Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

Interfering Substances:

Hemolysis: When hemolysis is present, the assay results should not be used. Hemolysis may contribute to error in assay results.

Reagents:

Preparation for Use: For Preparations B–D, reconstitute each vial of reagent with 0.01 mL of LD Isoenzyme Diluent.

Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

Interfering Substances:

Hemolysis: When hemolysis is present, the assay results should not be used. Hemolysis may contribute to error in assay results.

Reagents:

Preparation for Use: For Preparations B–D, reconstitute each vial of reagent with 0.001 mL of LD Isoenzyme Diluent.

Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

Interfering Substances:

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Reagents:

Preparation for Use: For Preparations B–D, reconstitute each vial of reagent with 0.0001 mL of LD Isoenzyme Diluent.

Storage and Stability: The vials should be stored at 2-6°C and are stable until the expiration date indicated on the vial. DO NOT REFRIGERATE OR FREEZE THE GELS

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Reagents:

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