Electrophoresis of Lactate Dehydrogenase Isoenzymes

Materials and Methods

Reagents

Electrophoresis

1. Electrophoresis buffer: 50 mmol of barbital and 0.94 mmol of EDTA per liter. Dissolve 10.3 g of sodium diethylbarbiturate, 0.35 g of the disodium salt of ethylenediaminetetraacetate (EDTA), 7 mL of 1 mol/L HCl in doubly distilled water to make 1 L of solution. The pH (at 20 °C) should be 8.6. Store this solution at 4 °C. Our practice is to use the buffer for two runs each day for five days, after which it is discarded.

2. Gel buffer: 64 mmol of barbital, 0.94 mmol EDTA, and 146 mmol of sucrose per liter. Dissolve 13.4 g of sodium diethylbarbiturate, 0.35 g of EDTA, and 50 g of sucrose, and dilute to less than 1 L with doubly distilled water: adjust the pH to 8.6 (20 °C) with a 1 mol/L solution of HCl, then dilute to 1 L.

3. Thin agarose film: This film may be cast by using the thin gel mold (fitted with linear wells) described by Elevitch et al. (2). The agarose [Indubiose A-37; Pharmindustrie (l'Industrie Biologique Française), Clichy, France] is prepared at 1 g of agarose per 100 mL of gel buffer (i.e., a 1% gel). The technique of making, and pouring, the gel is important for the preparation of satisfactory gels. First, place the gel mold and long Pasteur pipettes into an oven set at 45–50 °C. The agarose powder is mixed into a paste with a small amount of gel buffer. The correct amount of buffer is then added, and the mixture is heated over a flame or on a hotplate, with constant stirring, to or near boiling to dissolve the gel particles completely. The beaker containing the molten gel is placed in a hot water bath at 45–50 °C and allowed to cool with constant stirring (use a thermometer as the stirrer) until the gel temperature is just below 50 °C; then it is poured into the preheated (to 45 °C) gel mold through prewarmed long Pasteur pipettes. Elevitch et al. (2) suggest that the gel and gel mold should be chilled before removal of the gel attached to the lid from the mold.

Note: This formulation is also available commercially. The Universal Electrophoresis Film Agarose (Corning Medical, Medfield, MA 02052) is supplied in a pack containing two films, each sufficient for eight determinations. The mold enclosing each gel supplied by Corning may subsequently be used for casting new gels. The gel mold and lid must be thoroughly cleaned with ethanol, to remove grease along the edges, washed in detergent-containing water, rinsed well, and air dried. The edges of the mold should be given a light greasing with, e.g., Dow Corning High Vacuum Grease. The mold should be placed on a leveling table, the gel poured directly into the mold with slight overfilling, and the lid carefully lowered onto the mold, which will force out superfluous gel. The lid must not be allowed to spring back (otherwise bubbles will be sucked into the gel) but should be firmly held down with a heavy weight until the gel has formed.

Detection of LD isoenzymes

1. Lithium lactate: 0.5 mol/L, pH 7.0. Dissolve 2.4 g of

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Introduction

The occurrence of five lactate dehydrogenase (l-lactate: NAD+ oxidoreductase, EC 1.1.1.27; LD) isoenzymes in the tissues and extracellular fluids of the human, and the tetrameric structure of the LD molecule are now so well known that it is unnecessary to comment further on them.

Many techniques have been used for separating the LD isoenzymes. Electrophoretic methods on various media have been most popular, column chromatography has been used because of its relative speed, and a rapid immunological technique for the assay of LD-1 alone has recently been published. An exhaustive review, covering the literature up to 1969, is recommended for much useful information on this topic (1).

We describe here a method devised by Elevitch et al. (2) that requires 1 μL or less of serum and a thin-layer agarose preparation for electrophoresis. Detection of the LD isoenzymes is by fluorescence of NADH produced by the enzyme reaction. The method is accurate and precise, and requires a minimum of technologists’ time for work-up and performance.

Principle

At the pH used (8.6 at 20 °C), isoenzymes LD-1 to LD-4 migrate towards the anode and LD-5 moves towards the cathode on the agarose film. The LD isoenzymes are detected after a short incubation with a liquid overlay containing l-lactate and NAD+. The reaction

\[
\text{LD} \quad \text{l-lactate} + \text{NAD}^+ \rightarrow \text{pyruvate} + \text{NADH} + \text{H}^+
\]

produces NADH, which fluoresces at alkaline pH after excitation by light at about 360 nm. The isoenzymes are quantified by the relative proportion of fluorescence generated by each isoenzyme band.

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Proposed selected method

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L(+)-lithium lactate in doubly distilled water, adjust the pH to 7.0 (20 °C) with 100 mmol/L HCl, and dilute to 50 mL. Store at 4 °C for up to one month.

2. Substrate–coenzyme solution: 7.5 mmol/L β-NAD+ in lithium lactate solution. Dissolve 5 mg of β-NAD+ in 1 mL of the lithium lactate solution. Each agarose plate (eight estimations) requires 1 mL of this solution. Prepare this immediately before use, and discard any unused material.

Equipment
1. Model U77 electrophoresis tank with safety switch (Shandon Southern Instruments, Sewickley, PA 15143). The removable partitions in each electrode compartment are not required; cover the weighted bridge pieces with a single layer of Whatman No. 1 paper and set 8 cm apart to support the anodic and cathodic portions of the agarose plate.

2. Vokam constant voltage/current dc power supply (Shandon Southern Instruments).

3. Corning incubator-oven with removable incubation trays and drying shelves (Corning Medical).

4. Densicomp Model 445-50 fluorescent recording densitometer (Clifford Instruments Inc.). [This particular instrument has been replaced by the Model 750 scanning densitometer manufactured by Corning Medical. We have also used the Cliniscan Densitometer/Analyzer (Helena Laboratories, Beaumont, TX 77704).]

5. Ultraviolet light viewing box.


7. Oven set for 45–50 °C.

Collection and Handling of Specimens
The specimen (usually serum) must be maintained at room temperature because of the cold lability of LD-5 in serum (3–5). Serum may be stored at room temperature for three days, or possibly longer, without appreciable loss of activity. Heparinized plasma may be used, but fibrinogen must be removed by the addition of thrombin (about a spatula-tipful of bovine topical thrombin powder). Other body fluids may also require pretreatment with thrombin before separation of the LD isoenzymes.

This method can be satisfactorily applied to samples having an LD activity as low as one-half of the lower limit of normal serum LD activity. The activity of the sample should not exceed the upper limit of the normal serum LD activity, however, because substrate depletion over the most active LD isoenzyme bands will result in erroneous estimation of the isoenzymes (6). Similarly, if a substantial proportion (>70%) of LD activity resides in a single isoenzyme band, the sample should be diluted to the lower limit of normal serum LD activity before isoenzyme estimation (6).

Finally, as is well known, hemolyzed specimen should not be used for LD isoenzyme estimation because of the very high LD-1 and LD-2 content of erythrocytes; such specimens can be used where only the LD-5 isoenzyme is being measured, but only if LD-5 activity is expressed in absolute units, and not as a percentage of the total serum LD activity.

Procedure
1. Pour 1 L of electrophoresis buffer into the tank and tilt the tank so that the liquid in each compartment overflows the central partition. This ensures that the liquid levels in each compartment are equal. Remove any traces of buffer along the edge of the compartments to prevent the occurrence of a “salt bridge” between the electrodes. Ensure that the paper-covered bridge pieces are well soaked with buffer solution. The buffer should be at 4 °C and chilled enough for two separations to be completed, one after the other. Change the polarity after each run, if using the same buffer; thereafter, use another lot of chilled buffer. When the day’s separations are complete, return the buffer to cold storage.

2. Remove the agarose strip from the package, taking care to handle the film only by the edges. Each film has eight application wells towards the cathodic end. Before use, remove the top part of the plastic mold, used to cast the film, and leave the bottom part as a support.

3. Using the Drummond 1-μL Microcaps system, place approximately 1 μL of serum in the well nearest the anode. Replace the hard-rubber perforated cap provided with the system by a soft-rubber 3-mL dropper bulb (with no hole). This modification gives superior control during the pipetting process. Do not completely empty the Microcap because this may create a bubble in the application well. Reserve one position for quality-control serum or other control. Unless a jaundiced serum is available, dissolve a few crystals of bromphenol blue in one specimen before sampling to act as a migration marker.

4. Now place the loaded plate face down on the prepared bridge pieces of the electrophoresis tank. The application wells should be nearer the cathodic end of the tank. Electrophoresis is performed, under ambient conditions, at a constant voltage setting of 200 (i.e., 20 V/cm), which gives a current of 15–18 mA, for about 1 h. This time should be sufficient for the bromphenol blue marker (or jaundiced serum) to migrate about 33 mm anodally.

5. Remove the plate by holding its edges and carefully remove the buffer from the contact surfaces with a paper tissue. Lay the plate on a dry tissue and apply 1 mL (about 20 drops) of the substrate solution along the anode side of the plate and spread the solution evenly, with a single sweeping motion and no downward pressure, across the film to the cathode edge of the plate, back to the anode edge, and across the film once again with the side of a 5-mL pipette. Push excess substrate solution to, and off, the edge of the plate by this procedure, then wipe the edges of the plate dry with a paper tissue.

6. The agarose film is now ready for incubating. It is convenient to arrange that the incubator-oven is switched on before electrophoresis is commenced so that there are no delays in the subsequent processing of the plate. Prepare the incubator tray ahead of time by soaking the special paper liners with distilled water and allowing the chamber to equilibrate at 37 (±2) °C for at least 30 min. Place the agarose film, still contained in its plastic tray, on the bottom paper liner of the incubator tray. This liner must be wet enough to hold the plastic tray firmly by capillary action. Replace the incubator lid, with its paper liner, on the incubator tray and incubate for 15 min.

7. Carefully remove the agarose plate from the incubator tray, wipe the underside of the plastic tray dry, and place the agarose film, still supported in its tray, into the dryer shelf, agarose surface up. Attach the edges of the agarose film by rubber bands to the front and back of the dryer shelf. Place the shelf in the air oven and dry at 72 (±5) °C for about 15–20 min. Continue the drying until the agarose feels hard to fingernail pressure. The dried plate has a shiny surface; dull areas indicate inadequate drying. Do not overdry, because background fluorescence will increase to such an extent as to swamp the NADH fluorescence.

8. Before proceeding further, check the separation by viewing the agarose plate under ultraviolet light. Each separation should have five symmetrical blue-white fluorescent bands equidistant from each other, and no other fluorescent material should be visible. There also should be no background fluorescence from the agarose plate itself.

9. The individual LD isoenzyme separations can now be scanned with a fluorescent recording densitometer, and this
should be done within a few hours of drying. NADH is detected by illuminating the agarose plate with ultraviolet light at about 366 nm. Fluorescent emission above 405 nm is detected with a suitably filtered photodiode. Record the scan on paper. Separation is satisfactory if the peaks are symmetrical and the troughs between the peaks are within 1–2 cm of the baseline (maximum peak height should not exceed 15 cm). Draw the baseline for the scan by joining the zero fluorescent lines at the base of the LD-1 and LD-5 peaks.

Calculations

The peak heights above the ruled baseline are measured in millimeters and, with the total serum LD activity (U/L), are entered into a programmable printing desk calculator for the following calculations:

- the percentage activity of each isoenzyme
- the absolute activity (U/L) of each isoenzyme
- the percentage of monomeric components (i.e., H and M subunits)

The details of this calculation have been given (7).

With the Cliniscan process one can determine either peak heights or the area under the tracing, either of which can then be used to determine the percent activity represented by each peak. This capability considerably reduces the processing time associated with this stage.

Notes on Procedure:

Crystallization of substrate. The lactate/NAD+ substrate solution (step 5) can crystallize out on the agarose plate. We have found that the pH of the lactate solution should be kept as close to 7.0 as possible to avoid this.

Drying. Overdrying (step 7) will result in excess background fluorescence; therefore, the drying process must be carefully monitored so that the agarose plate is removed from the oven immediately, as soon as it is dry.

Bleaching of fluorescence. Experience with other densitometers in 1972 convinced us that over-illumination of the agarose plate during densitometry (step 9) will “bleach out” the NADH fluorescence and result in a very low fluorescent yield. Therefore, for satisfactory results, the intensity of the activating illumination may have to be reduced in models other than the one we have used, although there are now (1982) many densitometers commercially available that do not create this problem.

Results and Discussion

Quality Control

As with any procedure in a clinical chemistry laboratory, quality control of the total procedure is mandatory. We ordinarily include one quality control serum on each agarose plate (i.e., together with seven patients’ specimens), thus assuring ourselves and our clinical colleagues that each separation and quantification process is under scrutiny and is controlled.

We (8) have found that any commercial quality-control serum containing only human serum, with no additives, is suitable for the purpose of controlling the LD isoenzyme procedure. One caution is necessary, however: the serum chosen should have been lyophilized very promptly after collection because delay in this process appears to lead to asymmetrical isoenzyme peaks (8). One should thus screen the batch of potential quality-control serum to ensure that no artefacts are present.

Our procedure is to open a new vial of quality-control serum at the beginning of the week—or use the material available elsewhere in the laboratory (on the grounds of economy)—and use it until Friday, then discard it. The reconstituted serum is maintained at room temperature (18 °C) throughout the week. Examples of our results for “same-day” and “between-day” precision are given in Table 1.

Reference Intervals

Values were obtained for a population of healthy hospital personnel (n = 219). Table 2 lists values for percent of each LD isoenzyme and the proportion of H and M subunits.

Clinical Interpretation

The succinct account of this topic in its previous presentation as a Selected Method (7) is a valuable starting point, and Dito (9) has compiled an extensive dictionary of interpretative comments to be used as part of a simple reporting procedure. An interesting and very useful personal series of cases was collected by Zondag (3); his small book is highly recommended. The text by Batsakis and Briere (10) is also an extremely valuable source of information on the LD isoenzyme patterns in disease.

The most common abnormal patterns are increases in the anodic zone (i.e., LD-1 and LD-2) and in the cathodic zone (i.e., LD-4 and LD-5). Less commonly, mid-zone increases (LD-3) are seen. Normal patterns with above-normal total LD activity in the serum (the isomorphic increase of a normal LD isoenzyme pattern) are frequently observed and usually occur after multi-system involvement.

Increase in anodic isoenzymes (LD-1 and LD-2) are caused by hemolysis, ineffective erythropoiesis, renal infarction, germ cell tumors, or myocardial infarction. We stress, however, that hemolysis, either in vitro or in vivo, or ineffective erythropoiesis must always be considered as a possibility before assuming that heart muscle necrosis has occurred. For example, hemolysis caused by prosthetic heart valves is now seen very frequently; the ineffective erythropoiesis of megaloblastic anemia or thalassemia must also be kept in mind. Rarely, other causes of hemolysis may be responsible for the anodic pattern. Renal infarction and germ-cell tumors may also give an anodic pattern, although the germ-cell tumors can produce patterns showing a disproportionate increase (11) in LD-1 (Figure 1).

In myocardial infarction LD-1 in serum is increased but the extent of the increase, as reported in the literature, is quite variable. Using the present analytical system, we (12) have shown that:

- Diagnostically useful results can be obtained by reporting individual isoenzyme activities either as a percentage of the total activity or in absolute units of enzymic activity (Figure 2).
- “Flipping” of LD-1 does not occur, at any time, in at least 12% of all cases of myocardial infarction.
- The LD-1/LD-2 ratio, an alternative “test” for myocardial infarction, is abnormal by the present method when the ratio is above the reference range of 0.45–0.74. In a coronary care unit we found this test to have a sensitivity of 100% and a specificity of 90.5% (12).

Cathodic patterns suggest hepatic or skeletal muscle damage, and the reader is referred to Dietz et al. (7) for further details. Our own experience suggests that congestive cardiac failure is by far the most common cause of LD-5 increases in hospital patients. It is important to realize that the half-life of LD-5 in serum is only about 10 h (13), so that an increase in LD-5 may therefore be very temporary. By contrast, LD-1 has a half-life about 10-fold longer than this; thus LD-1 increases may be detected for many days after the release of the enzyme into the intravascular compartment. Irreversible congestive cardiac failure appears to be associated with LD-5 increases exceeding 70% of the total serum LD activity (14,
Table 1. Precision of LD Isoenzyme Assay

<table>
<thead>
<tr>
<th>LD isoenzyme</th>
<th>Same day, %</th>
<th>Between day, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>In normal human serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23.26</td>
<td>1.09</td>
</tr>
<tr>
<td>2</td>
<td>36.80</td>
<td>0.91</td>
</tr>
<tr>
<td>3</td>
<td>20.59</td>
<td>0.41</td>
</tr>
<tr>
<td>4</td>
<td>8.53</td>
<td>0.58</td>
</tr>
<tr>
<td>5</td>
<td>10.76</td>
<td>1.23</td>
</tr>
<tr>
<td>In commercial control serum: batch 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21.67</td>
<td>0.82</td>
</tr>
<tr>
<td>2</td>
<td>35.38</td>
<td>0.68</td>
</tr>
<tr>
<td>3</td>
<td>19.99</td>
<td>0.85</td>
</tr>
<tr>
<td>4</td>
<td>8.65</td>
<td>0.51</td>
</tr>
<tr>
<td>5</td>
<td>14.30</td>
<td>0.64</td>
</tr>
<tr>
<td>Batch 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23.3</td>
<td>0.64</td>
</tr>
<tr>
<td>2</td>
<td>37.6</td>
<td>0.78</td>
</tr>
<tr>
<td>3</td>
<td>22.1</td>
<td>0.80</td>
</tr>
<tr>
<td>4</td>
<td>6.8</td>
<td>0.58</td>
</tr>
<tr>
<td>5</td>
<td>10.0</td>
<td>0.66</td>
</tr>
<tr>
<td>Batch 3</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>23.3</td>
<td>1.11</td>
</tr>
<tr>
<td>2</td>
<td>35.6</td>
<td>0.93</td>
</tr>
<tr>
<td>3</td>
<td>22.4</td>
<td>0.51</td>
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<tr>
<td>4</td>
<td>8.2</td>
<td>0.61</td>
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<td>5</td>
<td>10.4</td>
<td>0.74</td>
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<tr>
<td>Batch 4</td>
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<td>1</td>
<td>22.3</td>
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<tr>
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<td>0.86</td>
</tr>
<tr>
<td>3</td>
<td>22.2</td>
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<tr>
<td>4</td>
<td>7.1</td>
<td>0.37</td>
</tr>
<tr>
<td>5</td>
<td>10.5</td>
<td>1.36</td>
</tr>
</tbody>
</table>

*From McKenzie and Henderson (8)*.

Table 2. Distribution of LD Isoenzymes in Normal Serum (n = 219)

<table>
<thead>
<tr>
<th>LD-1</th>
<th>LD-2</th>
<th>LD-3</th>
<th>LD-4</th>
<th>LD-5</th>
<th>LD-H</th>
<th>LD-M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>20.3</td>
<td>34.0</td>
<td>22.8</td>
<td>11.8</td>
<td>11.0</td>
<td>60.2</td>
</tr>
<tr>
<td>SD</td>
<td>2.80</td>
<td>2.26</td>
<td>1.59</td>
<td>1.75</td>
<td>2.49</td>
<td>3.00</td>
</tr>
</tbody>
</table>

An example of the increases that can be encountered in these patients is shown in Figure 3 with, for comparison, a pattern obtained from an autopsy specimen of liver.

Mid-zone increases (i.e., LD-3) are relatively rare, but are seen in cases of infective mononucleosis (i.e., lymphoid tissue involvement) and in any condition where platelets are rapidly destroyed (e.g., disseminated intravascular coagulation or massive blood transfusions). The so-called "malignant serum isoenzyme pattern"—an increase of LD-2, LD-3, and (or) LD-4—should also be included in this group, but this term must be used with caution: Zondag (3), for example, found the pattern to be present in only 46% of a group of patients with malignant disease. It should be appreciated, of course, that the term "malignant pattern" includes increases of serum LD-5 also.

A summary of these patterns is given in Table 3. Our own experience, in a tertiary teaching hospital, suggests that the LD isoenzyme pattern is largely used to rule out myocardial infarction and as a general screen when multi-organ pathology is suspected. It is incumbent on the clinical chemist to be aware of the several diagnostic possibilities of any LD isoenzyme patterns so that the physician requesting the test may be adequately advised by the laboratory. This aspect of isoenzyme interpretation has been stressed by Dito (9).

Artefacts appear to be very rare in this agarose system. However, there is one source of artefact for which the user must be alert. Patients with chronic renal failure on maintenance dialysis will show a sixth "LD-isoenzyme" band anodic to LD-1, apparently due to fluorescent albumin in this group of patients; the additional band can be detected by means of a simple blank reaction (17).

How accurate is this procedure? By "accuracy" is meant the agreement between the best estimate of a quantity and its true value (18). We have assessed accuracy by measuring the relative proportions of binary additions of semi-purified human LD isoenzymes (19, 20). Two pairs of binary additions were
used—the LD-1:L-5 and LD-1:L-2 combinations. The former system of additions established that no anodic (or cathodic) bias exists in the procedure (19) and the latter system proved that LD-1 and LD-2 are measured accurately (20). Thus LD-1/LD-2 ratios obtained by this system are without bias. This finding has considerable bearing on the influence

of hemolysis on the LD-1/LD-2 ratio. It has been claimed that hemolysis cannot invert this ratio (5) but our work (20) with this accurate LD isoenzyme procedure has shown that, if there is sufficient hemolysis and the erythrocyte LD-1 activity is greater than LD-2 activity (a common occurrence), the LD-1/LD-2 ratio will invert. Therefore hemolysis may be an important cause of false-positive results when the LD-1/LD-2 ratio is used as a test for myocardial infarction.

Addendum

We have had an opportunity to try the alternative methods for preparing the agarose plates brought to our attention by Evaluator C.M.E. and have found them extremely useful. These alternatives are outlined below.

Evaluators’ Comments

Evaluaton S.E.G.

General observations: I have compared the suggested

| Table 3. Summary of the Principal Causes of LD Isoenzyme Pattern Abnormalities |
|---------------------------------|---------------------------------|---------------------------------|
| **Anodic increases**            | **Mid-zone increases**          | **Cathodic increases**          |
| Infarction or surgery of myocardium | Rapid destruction of platelets | Liver diseases: congestive cardiac failure, early stages |
| In vivo or in vitro hemolysis | Lymphoid tissue involvement (e.g., infective mononucleosis) | of acute hepatitis, exacerbation of chronic hepatitis |
| Ineffective erythropoiesis | Splenic necrosis | Skeletal muscle injury |
| Germ cell tumors | Malignant pattern | Prostatic carcinoma |
| Renal infarction | Malignant pattern | Malignant pattern |
| Muscular dystrophies | | |
method with a dye-binding method. The proposed technique is more rapid, more easily performed, and in my hands, yields results with better precision.

Electrophoretic method: The Corning Universal Film was used with no difficulty. For buffers and reagents the directions of the proposed technique were followed with no problem. I found, however, that the electrophoretic buffer could easily be stored for one month at 4 °C.

I used the Corning/ACI Electrophoresis Tank with the matched power supply and performed electrophoresis in exactly 35 min. This consistently gave good separation of the five isoenzymes.

I found it very difficult to pipet 1 μL of serum accurately. I used, instead, a 10-μL pipette and an estimated 2–3 μL of sample per well. I also found it necessary to use this much serum to get adequate signal response.

Incubation and drying: An ordinary drying oven, set at the recommended temperature, was used with Corning incubator trays for the incubation. Drying was done in the same oven set at the higher temperature. After drying, there was some crystallization of buffer around the edges of the gel but never any in the pattern area. Further, under ultraviolet light, there was never any background fluorescence.

Gel scanning and results: Scanning the patterns presented some problems but this was apparently because of the lack of experience of the operator and was related to the spectrophotometer used. This question has not been adequately resolved. Within-run comparisons yielded CVs between 6 and 7% for peaks having about 10% of the total LD activity, and around 12% for peaks with activity less than that (n = 8). I was not able to reach that precision with the dye-binding technique.

Conclusion: I found the proposed technique to be rapid, easily performed, and reasonably precise, and am quite sure that with little effort it could be implemented in any laboratory desiring to perform electrophoresis of LD isoenzymes.

Evaluator L.M.E.

Preparation of plates: I do not believe that the method for preparing the gel mold described by Elevitch et al. (2) is suitable for use in a busy routine laboratory setting. Cutting and gluing plastic strips with model airplane cement is not appropriate to such a setting and in most hands will not provide precise enough wells. Injection of gel between warmed plain glass plates separated by a gasket and held together with spring clips is feasible. Sample application is then simply and precisely made by overlay of a thin re-usable template which has very fine slots cut in it, such as is supplied with Worthington Panangel plates. This technique gives narrower zones of applied sample and sharper resolutions of bands with less care required in making the sample application.

When re-using the Corning molds I used GelBond NF film (Marine Colloids Division, FMC Corp.), cut to size and held in position as an overlay with a spring clip at one end. As the gel was injected onto the mold, the GelBond film was then lowered gradually from one end so that all air bubbles were excluded. This procedure gives a plate similar to the Corning plates, with the gel adhering to the plastic film and the wells clearly indented in the upper surface of the gel when the mold is removed.

Collection and handling of specimens: The reference to storage (4) dates from 1964, when freezers were not so readily available: for example, it is stated that serum stored at −10 °C appeared not to be frozen. I agree that storage at 4 °C leads to changes in isoenzyme patterns and is not therefore a suitable temperature, but changes in pattern also occur at room temperature, and fast freezing to −20 °C seems the least likely to result in changes in composition before assay, if specimens cannot be assayed fresh.

It is noted that the activity of the sample for electrophoresis should not exceed the upper limit of the normal serum LD activity, to avoid substrate depletion. The optimal concentration of substrate cited by Buhl et al. (Clin. Chem. 23: 1289, 1977) is 50 mmol/L (albeit in a different buffer system), and these authors note that excess lactate inhibits LD-1. The substrate concentration used in this method is 500 mmol/L. Does the high lactate concentration account in any way for the low range of usefulness? Repletion of substrate during incubation by addition of several aliquots as suggested by the authors will alter substrate and cofactor concentrations with each addition because different amounts will have been used by each isoenzyme during these incubation periods. The claim that substrate depletion and repletion are the factors involved is open to question, and it would be preferable to start with optimized reaction conditions rather than have to resort to dilution of so many specimens. This whole phase of the method does not seem to be clearly worked out.

Care is required in removing all excess substrate to help prevent crystallization on drying. Even so, crystallization occurs sometimes around the periphery of the plates. Perhaps reduction of the lactate to a more optimal concentration would reduce the problem?

General comments: LD isoenzymes can be separated by the method described. The technique is not difficult to set up and perform, particularly with the use of commercially prepared plates. Use of home-poured plates makes the procedure longer, more tedious, and individual-technique dependent.
For use in routine general hospital settings where a variety of technologists may rotate frequently through the various work stations, the commercial plates would probably be preferred.

I did not have access to the recommended equipment, and other laboratory equipment was substituted. Under these conditions the method requires considerable care in its execution and depends on the skill and technique of the analyst. The fact that care is required is not by itself a deleterious feature, but it does mean that the method is less applicable to widespread use in routine laboratories, where technologists rotate frequently through the various work stations, than in those laboratories where one or two technologists are dedicated to this aspect of the work; this should also be borne in mind when selecting a method. Also, while I do not believe that a method should necessarily be excluded as a Selected Method because it is commercially available as a “system,” I believe it should be superior to other similar commercial systems before being “selected.”

It seems simpler to assay LD-1 by an immunological method than to perform electrophoretic separation of LD isoenzymes to rule out myocardial infarction. We find that this test is sensitive, if not completely specific, and if results for two samples drawn on admission and 24 h later do not show an increase, myocardial infarction can be ruled out.

Evaluator D.A.V.

Electrophoretic method: For convenience and because of a lack of experience of my technologists, we used Corning Universal Film rather than pouring our own plates. When we attempted to pour some plates, we had a great deal of difficulty with the instructions as presented in getting even, smooth plates; this led to artefacts when the plates were used. We also did not have the capability of measuring 1 µL of serum with any great degree of accuracy. As a consequence we found that using a 10-µL pipette and merely filling the wells on the plate until they were full worked very well. Because the amount of fluorescence was quite low and we had to use such a high intensity light, we further found that any reduction in this volume led to peaks that were almost indiscernible.

Results: Within-day CVs were between 0.69 and 1.53%. Between-day (day 1 and day 2 of analyses) were between 1.58 and 2.25%. The tracings themselves demonstrated clean peaks, resolved to or very near baseline.

General conclusions: The method was easy enough to follow and these tracings were done by at least three of the technologists in the laboratory as they rotated through the station. I made no attempt to train one individual, as I thought part of the evaluation was whether it could be put directly to use by technologists. The main problem appeared to be the time involved. It was considerably longer than the Helena system, with which we are more familiar. However, this increase of time very possibly could have been due to the unfamiliarity of the technologists with the technique and one might expect that if it were instituted in the laboratory, eventually the time involved would be reduced considerably. In general, I found the method to be a good method, offering a high degree of precision, considerably better than what I obtain with the dye-binding method we use. In addition, the clean separation of peaks and the lack of background fluorescence makes the patterns very easy to interpret. I believe that with a little practice, the method could be instituted in almost any laboratory, if modifications in specimen volume were made to provide sufficient fluorescence for instrumentation available for its measurement. The only real question I have is whether this degree of precision is necessary in a clinical situation to provide the necessary diagnostic information to the physician.

References


1 Ed. note: Guidelines proposed for Selected Methods in which commercial diagnostic products are used and where experts in industry are invited as Submitters or Evaluators have been published, for comment, in AACC’s Clinical Chemistry News (Sept. 1982, pp 7 and 18).

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