References

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Comments on the Proposed Selected Method for Electrophoresis of Lactate Dehydrogenase Isoenzymes

To the Editor:

We have read with much interest the Proposed Selected Method on the electrophoresis of lactate dehydrogenase (LD) isoenzymes (1) and have the following remarks:

1. We use a secondary filter with a cutoff at 455-nm. This gives a better signal-to-background ratio than does the 405-nm filter. With this 455-nm filter small scratches in the gel support do not disturb the results. The absolute peak height with this filter is lower, but this is easily overcome by using a higher electronic amplification.

2. We tried several commercial sources of NAD⁺. The Sigma Grade III, which is relatively cheap, does not produce a disturbing background fluorescence and is as good for its purpose as many more expensive brands of NAD⁺.

3. It cannot be stressed enough, that one should be aware of substrate depletion. To determine the amount of LD isoenzyme that can be measured accurately, we used a urine from a nephrological patient with a high LD activity, almost equally distributed over the five isoenzymes. Serial dilutions of this sample were electrophoresed, etc., as described by McKenzie and Henderson. We could thus establish up to which peak height (at a certain amplification) the response was linear. This height is the same for all five isoenzymes, and is an indication that the high molarity of lactate in this system does not produce specific inhibition of LD-1 (see comment by Evaluator L.M.E., page 194 in I). So we have a check afterwards if the percentage distribution is a real one.

4. We also distribute 1 mL of substrate solution over the gel; however, we found that using a single sweeping motion produces a gradient of lactate over the gel, resulting in earlier substrate depletion for LD-5 than for LD-1. With a 5-mL pipette we roll repeatedly from anode to cathode and back, until the fluid is completely absorbed by the gel.

5. We use the Pfizer incubator-oven. Drying sometimes is uneven; i.e., one part of the gel is wet, other parts are already dull. To overcome this, we apply, between the gel and the drying shelf, a copper plate of the same size as the gel. With a small volume of water we glue the gel to the plate, and hold gel, plate, and shelf together with elastic binders. We dry for 13 min, which vary seldom results in crystallization; after the 13 min we dry for about 2 min more with a hair-dryer.

6. We strongly recommend the method described by McKenzie and Henderson, because it is the only electroforetic method with a proven accuracy (determined by working with purified isoenzymes). However, it would be wise to set up a comparison of this system with other, formazan-based methods. Precision, accuracy, and the linearity range should be compared. Imahashi (2) did such a comparison in 1968. However, linearity and accuracy were not considered, which makes the comparison of little value.

7. In contrast to immunological methods, one can with this system quantify all isoenzymes. We, for instance, measure LD-4 and LD-5 in urine to localize urinary tract infections. Furthermore, the fluorescent method, being more sensitive than a formazan-based method, diminishes the time we have to spend concentrating the urine.

8. Storage of serum for longer periods (more than two weeks) was unsatisfactory: whereas in the one serum all the isoenzymes were stable, in another there was a rapid decrease of LD-4 and LD-5. We tried storage at different temperatures: 4, −20, and −70 °C, and in liquid nitrogen; we also tried stabilization with several agents—separately or in combination: NAD⁺, dimethyl sulfoxide, bovine serum albumin, ethylene glycol, and dithiothreitol. Even the recently described method (3) of stabilizing with glycerol was not satisfactory in our hands. In urine the isoenzymes are even more unstable: after storage for some days at −20 °C there was a complete loss of activity (except for a trace of LD-1). We measure urine the same day it is collected; after collection the sample is immediately chilled to 4 °C, at which temperature enzyme inactivation in urine is slower than at 37 °C or room temperature. Furthermore, LD-5 in urine is labile below a pH of 5.5.

References

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One of the Submitters replies:

Doris McKenzie (née Imahashi; see reference 2 above), to my great regret, left my Department a few years ago to help her urologist husband full-time, thus leaving me to answer observations like Dr. Appelmelk’s. His remarks are most interesting and will be valuable to possible future users of the method, especially his comments on urinary LD isoenzymes, with which I have no experience. Dr. Appelmelk has clearly perceived the need for a robust and accurate methodology to measure all LD isoenzymes. Incidentally, I have been rather saddened to observe the apparent indifference of the clinical enzymology community to questions of accuracy in isoenzyme work, and it is pleasant to have at least one articulate supporter!

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Editor’s note: These comments, and others concerning this and the other Proposed Selected Methods printed in Clinical Chemistry since 1977, are included in the latest compilation of these methods, Selected Methods of Clinical Chemistry, volume 10, now at the printer.