

Present day practice

Conditions for the estimation of serum lactic dehydrogenase activity using the LKB 3600 reaction rate analyser and the range found in healthy subjects

R. W. RICHARDSON AND C. P. PRICE
From the Biochemistry Department, Coventry & Warwickshire Hospital, Coventry

The LKB 3600 reaction rate analyser may be used to estimate serum lactate dehydrogenase activity (SLDH) by measuring the change in absorption at 340 nm due to a fall in concentration of reduced coenzyme.

The instrument is required to operate at at least 6° above the ambient temperature and is normally supplied set to operate at 35°.

Optimal conditions at this temperature were studied using sera from healthy subjects, from patients with proven myocardial infarction occur-

ring 48-60 hours previously, and from patients with non-obstructive jaundice. The isoenzymes were assessed by determining the ratio of SLDH to serum α hydroxy butyric dehydrogenase (Wilkinson, 1962).

Figure 1 shows that the peak of enzyme activity was found to be between coenzyme concentrations of 0.13 and 0.20 mM. It is possible that the different peak activities shown by some sera reflects the use of varying amounts of coenzyme to reduce endogenous pyruvate.

The optimal concentration of sodium pyruvate was found to be at 1.3 mM (Fig. 2) with at least 95% of maximum activity between 0.8 and 1.7 mM.

Suggested Procedure for Estimation of SLDH

REAGENTS

0.067 M phosphate buffer pH 7.4

Dissolve 1.780 anhydrous potassium dihydrogen phosphate (analytical grade) and 7.568 g anhydrous disodium hydrogen phosphate (analytical grade) in deionized water and make to 1 litre in a volumetric flask. Adjust pH to 7.4 with dilute hydrochloric acid or dilute sodium hydroxide.

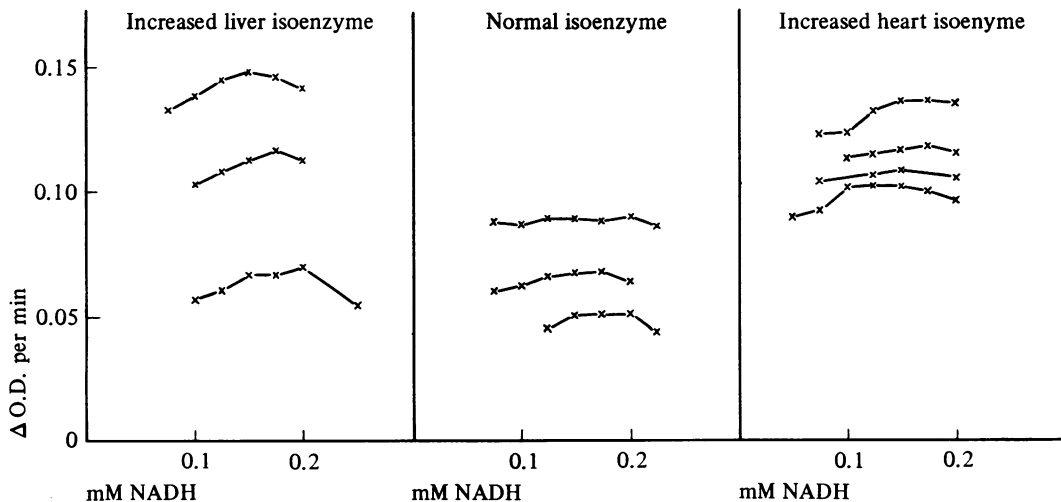


Fig. 1 Variation of LDH activity with coenzyme concn.ration using sera with different isoenzyme patterns.

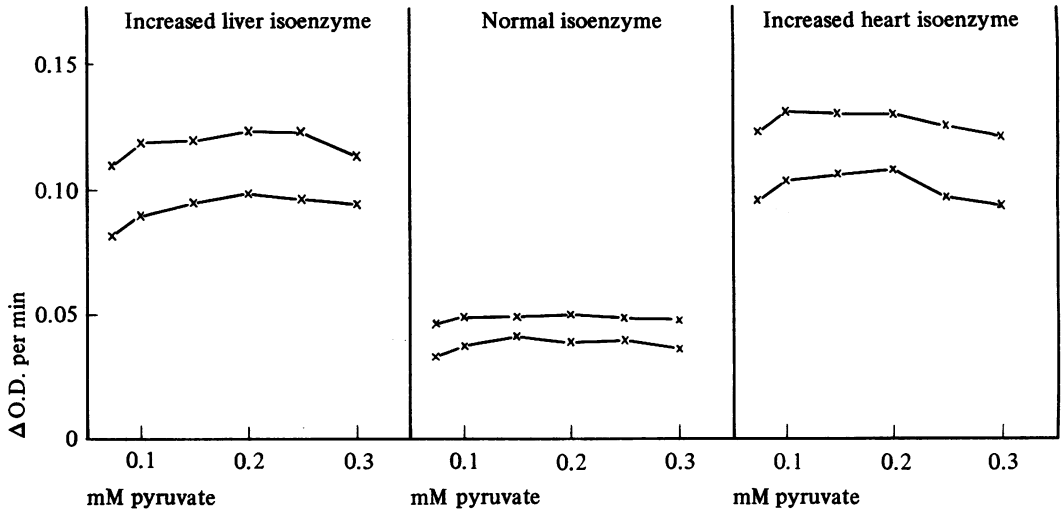


Fig. 2 Variation of SLDH activity with substrate concentration using sera with different isoenzyme patterns.

Reduced NAD 0.175 mM

Dissolve NADH (Sigma) in phosphate buffer in the ratio 1 mg to 8 ml buffer for use each day.

Sodium pyruvate 20 mM

Dissolve 220 mg sodium pyruvate (Sigma) in phosphate buffer and make to 100 ml in a volumetric flask. Store at 4°. Prepare freshly each week.

PROCEDURE

Pipette 1.40 ml reduced NAD solution into a cuvette. Add 0.05 ml serum. Mix and place in the rack. Insert into the reaction rate analyser that has previously been set to dispense 0.10 ml sodium pyruvate. Set background compensation to aperture 0.3 and measuring range with full scale deflection 0.2.

Use a measuring time of one minute and chart speed 50 mm/minute. Measure the rate of change of optical density.

$$\begin{aligned} \text{SLDH activity} &= \frac{1.55}{0.05} \times \frac{1000}{6.22} \times \frac{\text{change in OD}}{\text{per minute}} \\ &= 4984 \times \text{change in OD per minute.} \end{aligned}$$

An error of less than 0.3% is introduced by using the more convenient formula:

$$\text{SLDH activity} = \frac{\text{change in OD per minute}}{2}$$

× 10,000 milli International units per ml at 35°C.

Notes on the Suggested Method

1 Smaller quantities of reagents and sera may be used but the suggested method gives a convenient calculation.

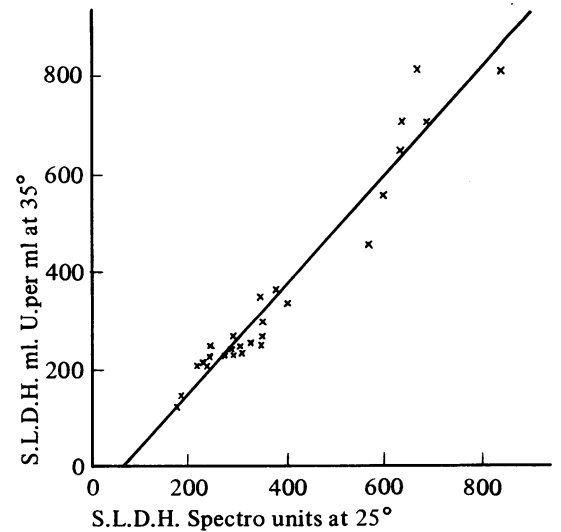


Fig. 3 Relationship between SLDH determined by the proposed method at 35° and the method of Wroblewski and La Due (1955) at 25°.

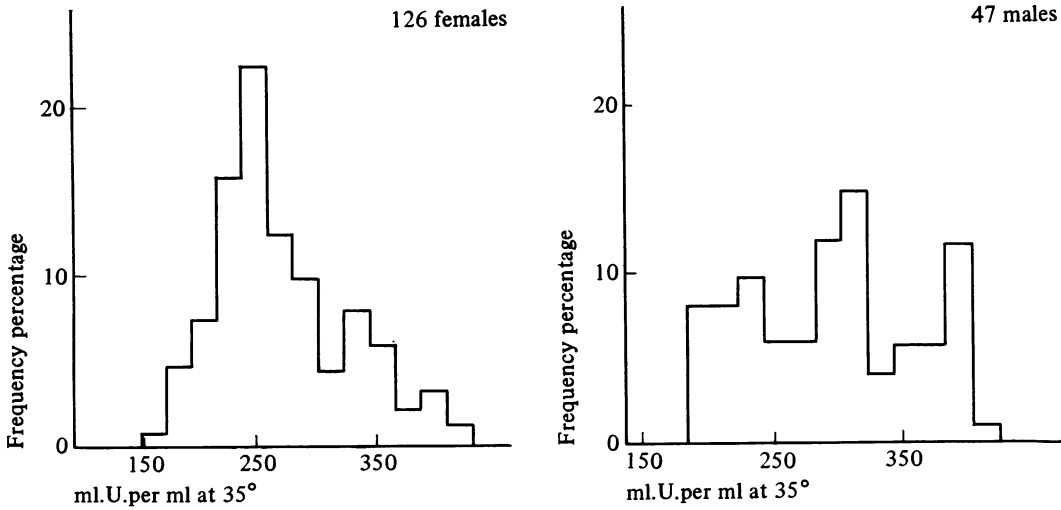


Fig. 4 Distribution of SLDH in a group of healthy adults.

2 The between-batch precision showed a coefficient of variation of 4.2%. Corresponding figures for the procedure previously used in this laboratory using the Optica recording spectrophotometer at 25° were 8.9% for SLDH.

3 A comparison of the results obtained by the method described and that of Wroblewski and La Due (1955) previously used in this laboratory was made by assaying 30 sera of varying LDH activity by both methods.

Figure 3 shows that the relationship can be expressed by the equation milli International units per ml at 35° = 1.09 × Units spectrophotometric at 25° - 71.

The results expressed in milli International units per ml at 35° using the LKB 3600 are therefore numerically close to those obtained in spectrophotometric units at 25°. This avoids confusion as clinicians do not need to adjust to completely different figures.

The range found in healthy adults was determined using blood samples drawn from 47 healthy male

and 126 healthy female volunteers (age range 16-62 years). The subjects were taken from various grades of hospital staff. No clinical examination was undertaken. Staff working normally who did not admit to illness on questioning were assumed to be healthy for this purpose.

Figure 4 shows the distribution of SLDH activity found. The distribution was approximately Gaussian when the frequency was plotted against the logarithm of the SLDH activity. Ranges found were:

SLDH	Range
47 healthy males at 35°	Mean 295.7 m IU per ml 95% limits 195-420
126 healthy females at 35°	Mean 275.7 m IU per ml 95% limits 185-390

References

Wilkinson, J. H. (1962). *An Introduction to Diagnostic Enzymology*. Arnold, London.
 Wroblewski, F., and La Due, J. S. (1955). Lactic dehydrogenase activity in blood. *Proc. Soc. exp. Biol. (N.Y.)*, 90, 210-213.