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This Week's Citation Classic®

Henry R J, Chiamori N, Golub O J & Berkman S. Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic acid dehydrogenase.
Amer. J. Clin. Pathol. 34:381-98, 1960.
[Bio-Science Research Foundation, Bio-Science Laboratories, Los Angeles, CA]

Lactic acid dehydrogenase, glutamic-pyruvic acid transaminase, and glutamic-oxalacetic acid transaminase of serum and their determination by spectrophotometric measurement of the rate of oxidation of DPNH were studied. Variations in substrate concentration, pH, and buffer concentration were examined. Studies were made of the relation between room and cuvet compartment temperature in Beckman Model B and DU spectrophotometers and of the relation between compartment temperature and temperature of the cuvet itself. Revised spectrophotometric methods were subsequently proposed, and adult normal values for the serum and cerebrospinal fluid enzymes were obtained using these methods at an incubation temperature of 32° C. [The *SCI*® indicates that this paper has been cited in over 510 publications.]

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Examination of the various methods proposed for the determination of lactic acid dehydrogenase, glutamic-pyruvic transaminase, and glutamic-oxalacetic transaminase that had been published in 1955[1] and 1956[2,3] revealed wide variations in substrate concentrations used and showed that, in some instances, no attempt was made to control the temperature of the assay.

These tests had already become important tools to the physician in the diagnosis of myocardial infarction as well as several other conditions. We recognized the urgent need for a thorough study of optimal concentrations of substrate, buffer, and pH and of temperature control. The methods that were studied all measured the rate of oxidation of reduced diphosphopyridine nucleotide (DPNH) by following the decrease in absorbance at 340 mμ. There was a need for a stable reference blank against which the decrease in DPNH absorbance at 340 mμ could be measured. This was provided by using a solution of potassium dichromate acidified with sulfuric acid.

The two instruments capable of measuring absorbance at 340 mμ and easily available in this country at that time were the Beckman DU and Beckman B. An attachment was available for the DU instrument to control the cuvet compartment temperature but not for the model B. The attachment was used with the model DU and thermistors were installed in both instruments to monitor temperature. For a number of reasons, the precisions of the assays attainable with the B never were as good as those attainable with the DU.

We were successful in altering the test procedures to give optimal conditions for the enzyme reactions. In those cases where temperature control was not available, as long as the reaction temperature was known the final results could be corrected to the standard temperature of 32° C, which was arbitrarily chosen. Variations in pH and buffer concentration were also studied and standardized.

It is my opinion that this publication has been so frequently cited because it became the first source for optimally standardized methods for these three enzymes and hence was, for a long period of time, the reference against which other proposed methods were compared. Furthermore, these methods were frequently used in clinical studies where one or more of these assays were employed.

[For recent advances in these methods, see references 4 and 5.]

1. **Wroblewski F & La Due J S.** Lactic dehydrogenase activity in blood. *Proc. Soc. Exp. Biol. Med.* 90:210-13, 1955. (Cited 1,795 times.)
2. **Steinberg D, Baldwin D & Ostrow B H.** A clinical method for the assay of serum glutamic-oxalacetic transaminase. *J. Lab. Clin. Med.* 48:144-51, 1956. (Cited 110 times.)
3. **Wroblewski F & La Due J S.** Serum glutamic pyruvic transaminase in cardiac and hepatic disease. *Proc. Soc. Exp. Biol. Med.* 91:569-71, 1956. (Cited 800 times.)
4. **Vanderlinde R E.** Measurement of total lactate dehydrogenase activity. *Ann. Clin. Lab. Sci.* 15:13-31, 1985.
5. **Mizutani F, Sasaki K & Shimura Y.** Sequential determination of L-lactate and lactate dehydrogenase with immobilized enzyme electrode. *Anal. Chem.* 55:35-8, 1983.

