A rapid blood lactate assay using a centrifugal analyser and 3-acetylpyridine-adenine dinucleotide

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It is being increasingly recognised that major forms of lactataemia and lactic acidosis are of clinical importance because they are amenable to treatment (Krebs et al., 1975; Cohen and Woods, 1976). However, the diagnosis of lactic acidosis demands the routine availability of a robust, simple, and rapid lactate assay. That no such entirely satisfactory method at present exists is amply indicated by the ever increasing literature on lactate assays, each emphasising convenience, speed, and simplicity.

Of recent note is the demonstration that fluoride-stabilised plasma is a satisfactory specimen for lactate analyses (Westgard et al., 1972), and that lactate determinations are possible with an electrochemical enzymatic sensor (Racine et al., 1975; Durliat et al., 1976), or with ferricyanide and lactate dehydrogenase from yeast (Durliat et al., 1976), or by the conventional NAD and lactate dehydrogenase assay using reaction rate techniques on a centrifugal analyser (Pesce et al., 1975) or miniature centrifugal analyser (Hadjiiannou et al., 1976). The emphasis in each case is on convenience and speed. We describe a rapid, ratiometric, lactate assay, using a centrifugal analyser, based on the reaction involving 3-acetylpyridine-adenine dinucleotide (APAD), instead of NAD, which has been described by Holzer and Söling (1965) and Maurer and Poppendiek (1974). The advantage of this approach is that the APAD/APADH system has a more positive redox potential than the NAD/NADH system and therefore the product of oxidation, pyruvate, does not require trapping. The test formulation is therefore simpler.

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Material and methods

CHEMICALS

3-Acetylpyridine-adenine dinucleotide (APAD), supplied by P. L. Biochemicals Inc, Milwaukee, Wisconsin. Lot number 327001 (94% pure) was used throughout the entire project. A 13·5 mmol/l solution, in doubly distilled water, was prepared freshly each day.

Lactate dehydrogenase from rabbit muscle. Three formulations were used:

1. Crystalline suspensions in 3·2 mol/l ammonium sulphate suspension having a specific activity of about 500 U/mg protein supplied with the Lactate Test Combinations by BMC Diagnostics/Biochemicals Ltd, St. Laurent, Quebec. Stable for many years at 4°C.
2. Lyophilised, salt-free powder having a specific activity of about 400 U/mg protein supplied by Sigma Chemical Co, St. Louis, Missouri. 5 mg was dissolved in 6·8 mol/l d-glycerol. Stable for one month at 4°C.
3. Same lyophilised material described above dissolved in doubly distilled water. Prepared freshly each day.

Glycine-EDTA buffer 1 mol/l glycine containing 5·37 mmol/l disodium EDTA. pH adjusted with 1 mol/l NaOH. Stable for one week at 4°C.

Working buffer mixture 1 ml of the lactate dehydrogenase preparation was added to 12 ml of the glycine-EDTA buffer.

Working lactate standard L-(+)-lactate, lithium salt, supplied by ICN Nutritional Biochemicals, Cleveland, Ohio. 6 mmol/l solutions were prepared with doubly distilled water. Aliquots were frozen at −20°C and were found to be stable for at least two months.

ENZYME ASSAY

Lactate dehydrogenase activity was assayed by the method of Tuckerman and Henderson (1973) at 37°C.
**SPECIMEN PREPARATION**

Blood was obtained by the technique of Ewen and Henderson (1976). The acid mixture used was 10 g/dl trichloroacetic acid in 0-5 mol/l HCl (Gloster and Harris, 1962). The clear supernatant obtained after centrifugation was either analysed immediately or stored frozen at 20°C until analysed. The weight of blood was derived from the weights of the collecting tube before and after collection.

**SPECTROPHOTOMETRIC ASSAY FOR LACTATE**

A centrifugal analyser, supplied by Electro-Nucleonics Inc, Fairfield, New Jersey, was used.

After diluting the acid extract of blood 1:2 with doubly distilled water, 25 μl of sample, water (for a reagent blank), and standard were aspirated and flushed with 350 μl of the buffer-enzyme mixture into the C-wells of the transfer disc using the rotor loader. (Cuvette 1 is the water-reagent blank, cuvette 2 the standard, and cuvettes 3-16 are tests.) 125 μl of the APAD solution is dispensed automatically into the B-wells. The instrument settings are: temperature 30 or 37°C; wavelength 363 nm; mode ‘end point’; running mode ‘auto’; first reading 300 s (30°C) or 180 s (37°C); number of readings 1.

The programme automatically blank corrects using cuvette 1, and the lactate concentration is calculated from the standard absorbance in cuvette 2 (ie, ratiometric).

**CALCULATION OF RESULTS**

The blood levels of lactate were obtained by correcting for the dilution factor and using the average male and female specific gravity of blood (Phillips et al., 1950).

**Results**

By examining the linearity of response to standard lactate concentrations up to 8 mmol/l, the following optimum reagent conditions were established:

- **Glycine-EDTA buffer** 1 mol/l, pH 9-6 (20°C)
- **Lactate dehydrogenase** 2000 U/l; not to fall below 1500 U/l
- **APAD** 13-5 mmol/l; not to fall below 10 mmol/l.

The formulation of the lactate dehydrogenase preparation proved to be critical. We initially used the 3.2 mol/l ammonium sulphate suspension but this was found to block the Micromedic pump valves, causing leaks and gross errors in pipetted volumes. We also found that it was difficult to obtain aliquots of similar enzyme activity from the enzyme suspension as it was usual to obtain a 50% difference in enzyme activity on sequential sampling. As the completion of the reaction is critically dependent on the amount of enzyme activity present, this formulation was therefore deemed unsatisfactory.

The 6.8 mol/l glycerol solution of the lyophilised enzyme was free of these disadvantages although it had considerably less storage stability. However, it was finally found more convenient just to use an aqueous solution of the lyophilised preparation although it had to be prepared daily.

Maurer and Poppendiek (1974) added a small volume of 2 mol/l potassium bicarbonate to the reaction mixture, presumably to neutralise the acid extract of blood. We examined the effect of adding sample volumes containing 0-10 g/dl trichloroacetic acid on the linearity of response using the above-mentioned assay conditions. It was found that only above 7 g/dl was there any inhibition of the reaction, and this was about 20% at 1 mmol/l and less than 1% at 8 mmol/l lactate. At the nominal maximum concentration of trichloroacetic acid used (5 g/dl), no inhibition was encountered, and it was therefore concluded that the bicarbonate was not necessary.

The linearity of response of this system is shown in Figure 1. The spectrophotometric response is linear up to at least 8 mmol/l of L-lactate in the standard solution. This is equivalent to a blood level of approximately 16 mmol/l (because of the approximate 1:1 dilution of the blood on collection). This range is satisfactory for all but the most severe cases of lactate acidosis.

![Lactate standard curve](image)

**Fig. 1** The linearity of response of the assay. Because of the approximate 1:1 dilution of the blood sample, the linear response is therefore up to, at least, 16 mmol/litre of blood lactate.
Technical methods

Precision studies (n = 25) gave coefficients of variation of (within-batch analyses) 1·5%, 1·6%, and 1·2%, and (between-batch analyses) 3·8%, 2·0%, and 1·1% for blood levels of, respectively, 2, 7, and 12 mmol/l lactate.

Recoveries of 102% (coefficient of variation of up to 2·4%) were obtained for whole blood into which additions of 0·5-6·5 mmol/l of lithium lactate had been made.

Comparison (n = 18) between the present method and the Boehringer-Mannheim kit for lactates (using the lyophilised enzyme) gave a regression line of \( y = 1·041x - 0·162 \) (the commercial method being the abscissa) with a correlation coefficient of 0·999 (Fig. 2).

Normal values obtained on 27 fasting laboratory staff gave a range of 0·38-1·28 mmol/l with a median value of 0·67 mmol/l (the distribution being positively skewed).

Discussion

The Table gives a comparison between the two published methods and the present method. By raising the temperature of assay, it is evident that the reaction proceeds much faster and less sample is required. On the other hand, the present method requires about six times more APAD than the original method of Holzer and Söling (1965). However, the gain in the speed of analyses (of about four to five times) with the resulting, more efficient, utilisation of technologists’ time clearly offsets this disadvantage.

We have preferred to use an acid extract of blood as such a preparation has a longer stability and it can be used for the analysis of other metabolites whereas this may not be true for the fluoride-treated plasma sample. Certainly, the fluoride specimen is easier to collect but we believe that the slightly extra inconvenience (for the physician) of collecting the specimen in acid should eliminate many of the less essential requests.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of methods using APAD for lactate determination</th>
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<tbody>
<tr>
<td>Glycine buffer (mol/l)</td>
<td></td>
</tr>
<tr>
<td>Working solution</td>
<td>1·0 pH 9·5</td>
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<tr>
<td>Assay concentration</td>
<td>0·78</td>
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<tr>
<td>APAD</td>
<td></td>
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<tr>
<td>Working solution (mmol/l)</td>
<td>7·16</td>
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<tr>
<td>Assay concentration (mmol/l)</td>
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<td>Amount used per assay (mmol)</td>
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<td>Total volume of assay (μl)</td>
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<tr>
<td>Sample volume (μl)</td>
<td>up to 40</td>
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<tr>
<td>Volume of blood required (μl)</td>
<td>approx. 10</td>
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<tr>
<td>Time to completion of reaction (min)</td>
<td>15-20</td>
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<tr>
<td>Temperature of assay (°C)</td>
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</table>

References


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**Letter to the Editor**

**Pseudomembranous colitis**

Price and Davies (1977) suggest that capillary microthrombi are unlikely to be the initiating cause of pseudomembranous colitis. Their evidence appears to be based on the absence of such capillary microthrombi in the 13 cases described as 'type I lesion' in their material.

In a recent study undertaken in our department (Bogomoletz, 1976), the presence of capillary fibrin thrombosis was demonstrated in rectal biopsies from five patients with clindamycin-associated colitis, three with pseudomembranous colitis, and two with non-specific colitis (the latter form appears to be an even earlier lesion than 'type I lesion').

Furthermore, capillary thrombosis with vasculitis has been described in rectal biopsies of other cases of clindamycin-associated colitis (Butsch et al., 1975).

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**References**


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