Analytical Requirements for the Measurement of Lactate

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The aim of this note is to throw some light on the analytical aspects of lactate determination. The sample material (blood or plasma), the mode of sample preparation (none, hemolysis, deproteination), and the selected reaction pathway all exert an influence on the final result (Fig. 1). An absolutely true lactate value is therefore not existing.

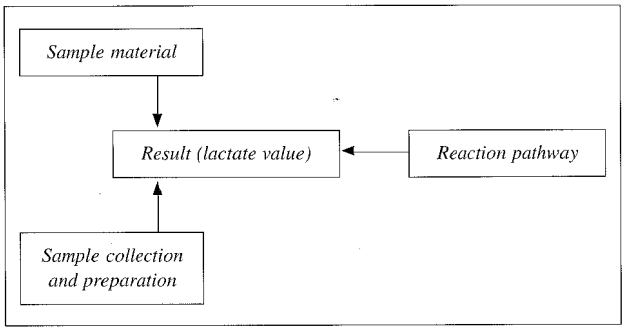

Figure 1:
Different influences on the result of lactate determination

First, the method of determination

There are various reaction pathways for the measurement of lactate, which differ in their reaction principle and in the result they produce (Fig. 2). A distinction is made between enzymatic photometric methods [1] and enzymatic electrochemical methods. The decisive reaction step is always the conversion of lactate into pyruvate by oxidation or dehydrogenation. This can be done with LDH and NAD^+, as shown in example 1; the increase in NADH is then measured in UV, as in the test combination for sports medicine from Boehringer Mannheim (BM Sportkit). Another pathway is oxidation with oxygen and LOD (example 2); the hydrogen peroxide formed as intermediate is oxidized further at an electrode and the flow of electrons is measured. This measurement principle is used, for example, in the instrument manufactured by Yellow Springs Instruments (YSI) [2]. However, it is also possible to transfer electrons with an electron-carrier or mediator, which in turn, in a second step, undergoes a redox color reaction (example 3); the dye formed in the last step is then used for measurement, as e.g. in the Accusport® lactate system.

Example 1:

$$\text{L-lactate} + NAD^+ + OH^- \xleftrightarrow{\text{LDH}} \text{pyruvate} + \underline{\textbf{NADH}} + H_2O$$

$$\text{pyruvate} + O_2 \xleftrightarrow{\text{GPT}} \text{L-alanine} + \alpha\text{-ketoglutarate}$$

Example 2:

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOD}} \text{pyruvate} + \mathbf{H_2O_2}$$

$$\mathbf{H_2O_2} \xrightarrow{\text{Pt anode}} O_2 + 2H^+ + \underline{2e^-}$$

Example 3:

$$\text{L-lactate} + \text{mediator}_{\text{form I}} \xrightarrow{\text{LOD}} \text{pyruvate} + \textbf{mediator}_{\textbf{reduced}}$$

$$\textbf{mediator}_{\textbf{reduced}} + \text{2,18-phosphomolybdate} \longrightarrow \underline{\textbf{molybdenum blue}} + \text{mediator}_{\text{form 2}}$$

Figure 2:
Various reaction pathways for the analytical determination of lactate

Sample preparation

In addition to the above methodological differences mention should be made of the question of application; the collection of samples for the determination of lactate is no easy matter since, during exercise, the values change considerably within a very short time (Fig. 3). Collection of a blood sample from the earlobe with its subsequent deproteination in perchloric acid has proved successful in this connection. Samples obtained in this way are stopped immediately; they remain stable for several days, and can be measured later in a laboratory. However, the resulting lactate values represent the sum of the lactate in plasma and in erythrocytes, because the latter are destroyed by the deproteination step.

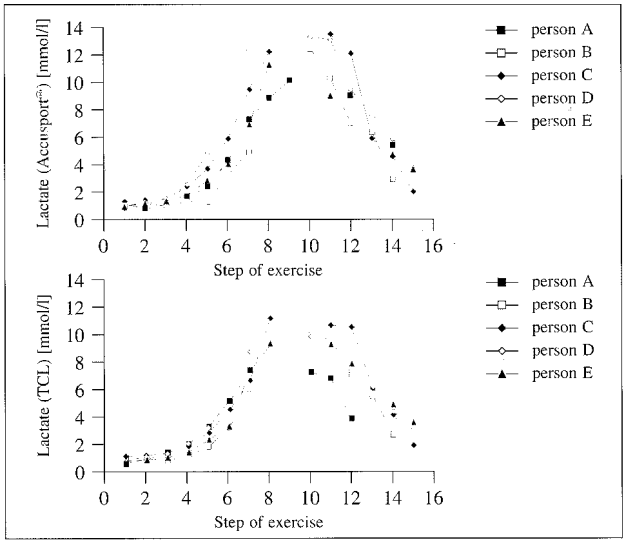

Figure 3:
Changes of lactate values during exercise

Nevertheless, even methods which use the same sample material give different results. Figure 4 compares the Behring Testomar® testkit with the BM Sportkit. Differences are found between these methods, particularly in the higher concentration range; Passing-Bablok regression yields a slope of 0.947, i.e. the measurements with the Testomar® testkit are on average 5% lower.

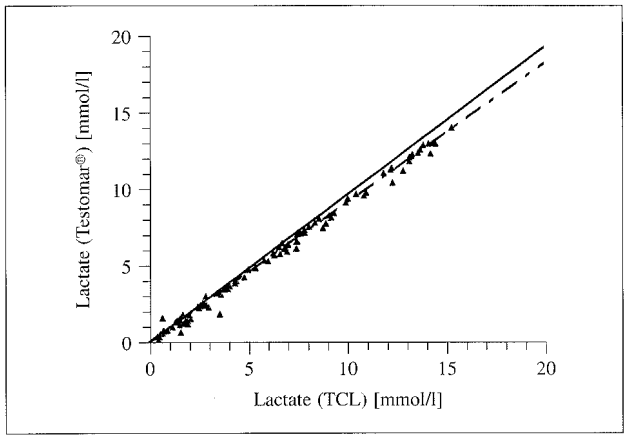

Figure 4:
Comparison Testomar® testkit versus Test Combination Lactate for Sports Medicine (TCL); Passing/Bablok regression: $y = 0.025 + 0.947x$; $n = 102$; $r = 0.997$

Even with one and the same method serious differences in performance can be encountered between one laboratory and the next. Figures 5 and 6 compare the ESAT® analyzer (Eppendorf) and the BM Sportkit methods. Whereas in the first example there is near-agreement and only a narrow range of scatter, in the second there is a mean methodological difference of 32% and wide scatter. Passing-Bablok evaluation leaves open the question, which of the two methods is responsible for the scatter and the difference.

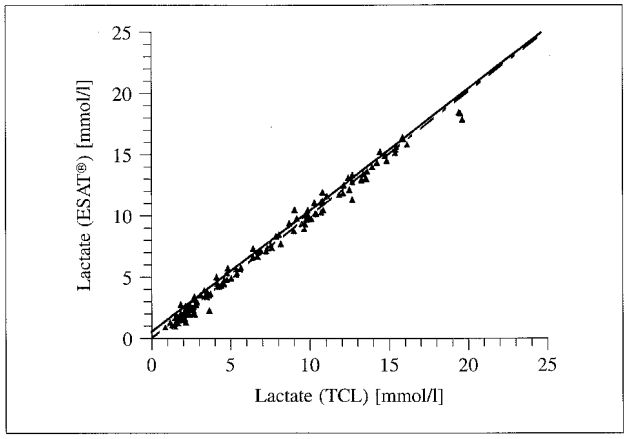

Figure 5:
Comparison ESAT® analyzer versus Test Combination Lactate for Sports Medicine (TCL), lab 1; Passing/Bablok regression: y = – 0.651 + 1.022x; n = 139; r = 0.995

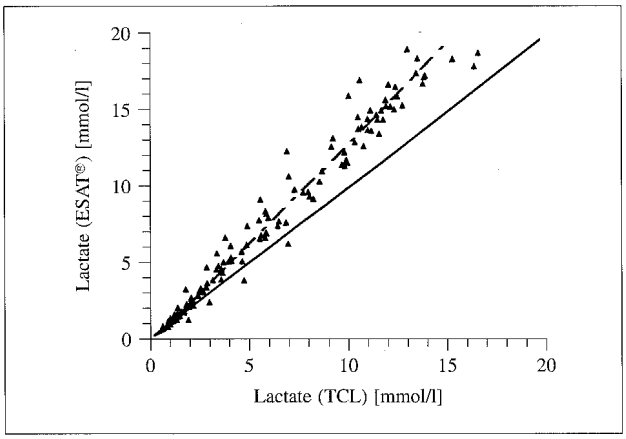

Figure 6:
Comparison ESAT® analyzer versus Test Combination Lactate for Sports Medicine (TCL), lab 2; Passing/Bablok regression: y = – 0.219 + 1.321x; n = 139; r = 0.985

Sample material

Figure 7 shows a comparison of the YSI analyzer and the BM Sportkit method. With a moderate range of scatter, the measurements obtained with the YSI analyzer are approximately 20% lower than those with the BM Sportkit. A further problem with lactate measurement becomes evident here – one which relates to the sample material. Lactate is present in different concentrations in the different blood compartments, i.e. in the erythrocytes and plasma. The YSI system electrode measures lactate in untreated blood – i.e. predominantly in plasma, but plasma which is "diluted" by the still present erythrocytes. The erythrocyte contents are inaccessible to the electrode, so the value of this so called "actual plasma" is always lower than that in deproteinated whole blood. The advantage of this method, however, is that a result can be obtained relatively quickly: in 1–2 min as opposed to 30 min by wet chemical methods [3].

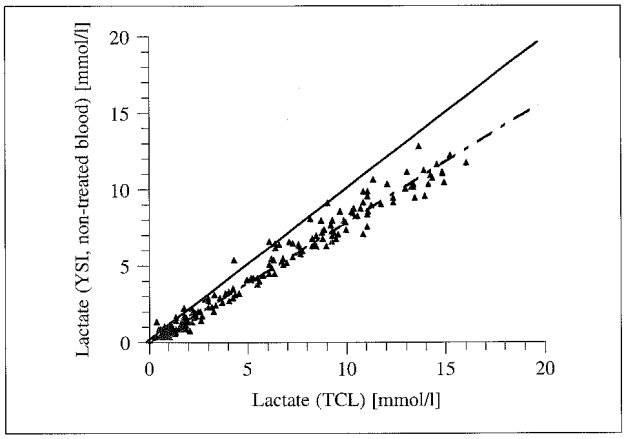

Figure 7:
Comparison Yellowsprings Instruments analyzer (YSI) versus Test Combination Lactate for Sports Medicine (TCL); Passing/Bablok regression: y = – 0.271 + 0.804x; n = 193; r = 0.986

Now to the Accusport® system

The Accusport® system is a quick drychemistry based lactate system which displays a result within 1 min. Its design and the reaction equation are shown in Figures 8 and 9. The topmost element of this layered structure is a yellow mesh, which represents the area where the blood is applied; next comes a glass-fiber layer for separation of the erythrocytes, followed by the actual reaction layer, in which the lactate is oxidized to pyruvate with the aid of lactate oxidase (LOD). The reaction layer is measured from below. A mediator transfers electrons from lactate to phosphomolybdic acid, which then forms the dye molybdenum blue, which in turn is measured by reflection photometry. Although the Accusport® system, like the YSI system, employs direct application and measurement of a drop of blood from the earlobe or fingertip, it differs from the YSI system in that is does not measure lactate in actual plasma. Rather, the erythrocytes are separated off by the glass-fiber layer prior to the reaction, which means that the measurement is carried out in plasma.

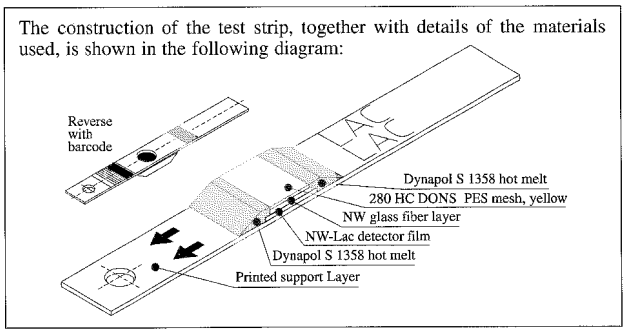

Figure 8:
Construction of the Accusport® test strip

Reaction principle

L-lactate + mediator$_{form\,1}$ $\xrightarrow{LOD}$ pyruvate + **mediator$_{reduced}$**

mediator$_{reduced}$ + 2,18-phosphomolybdate $\longrightarrow$ **molybdenum blue** + mediator$_{form\,2}$

Explanatory notes

The mediator transports the electrons from L-lactate to 2,18-phosphomolybdate. In the first step **form 1** oxidizes L-lactate with the aid of lactate oxidase (LOD) and is itself reduced in the process. The reduced mediator reduces 2,18-phosphomolybdate to the blue dye molybdenum blue and is itself oxidized in the process. However, oxidation of the reduced mediator produces not the starting compound, but another compound, here referred to as **form 2**.

Figure 9:
Reaction principle of the Accusport® test strip

Since it is known that different values are obtained from lactate measurements performed in plasma and hemolyzed or deproteinated blood [1, 4, 5], it was necessary to clarify the correlation between the two values so that, if possible, the result could be displayed in either way depending on the user's choice. Nevertheless, the method which uses deproteinated whole blood has acquired a certain standard status, and sports doctors have very many empirical values based on this sample material. A study in 41 male and female subjects of differing ability was therefore carried out to determine the so-called plasma-to-blood factor (Fig. 10), which also makes it possible to give whole blood values with the Accusport® lactate system, providing a link with the already existing database. If the data in the lower concentration range are examined more closely, it is seen that the factor changes as function of concentration (Fig. 11).

All this has been taken into account, and on the basis of the above findings an attempt has been made to reflect the pre-analytical and methodological differences in the Accusport® lactate system for practical use.

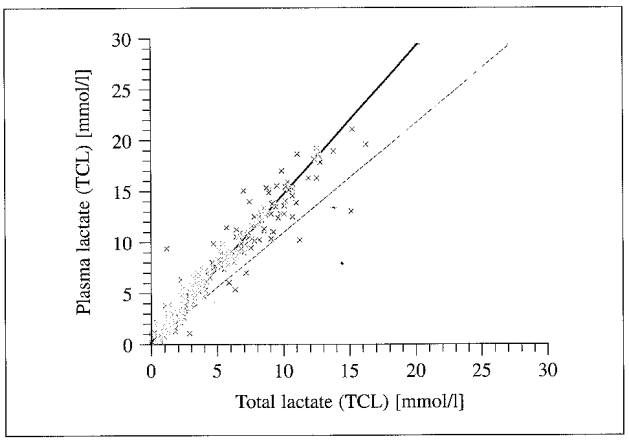

Figure 10:
Comparison plasma lactate versus total lactate with Test Combination Lactate for Sports Medicine (TCL); Passing/ Bablok regression: y = −0.085 + 1.350x; n = 288; r = 0.967

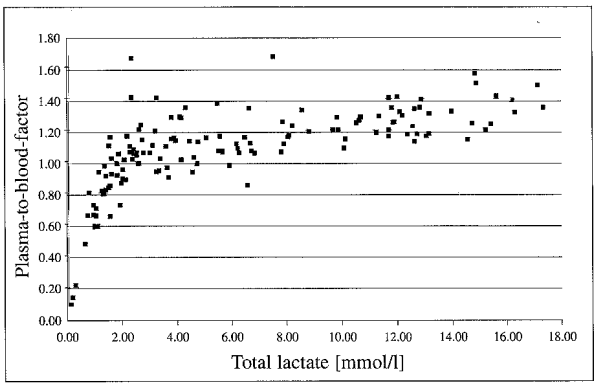

Figure 11:
Concentration dependency of the plasma-to-blood-factor

References

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