

Assessment of blood lactate: practical evaluation of the Biosen 5030 lactate analyzer

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ABSTRACT

DAVISON, R. C. R., D. COLEMAN, J. BALMER, M. NUNN, S. THEAKSTON, M. BURROWS, and S. BIRD. Assessment of blood lactate: practical evaluation of the Biosen 5030 lactate analyzer. *Med. Sci. Sports Exerc.*, Vol. 32, No. 1, pp. 243–247, 2000. **Purpose:** The aim of this study was to assess the validity and reliability of the Biosen 5030 lactate analyzer compared with a YSI 2300 lactate analyzer and a Kodak Ektachem DTII in a practical laboratory study context. **Methods:** To assess validity, 144 triplicate capillarized blood samples, across a range of values, were analyzed using the three analyzers. To assess reliability a further 665 samples were repeat analyzed. Temporal stability was determined by the reanalysis of resting and maximal exercise blood samples, after a period of storage ranging from 7 to 20 h, at room temperature. To measure inter- and intra-investigator reliability, 20 resting samples were taken from three different subjects by different investigators and a coefficient of variation was determined. **Results:** There were strong relationships between the Biosen, the YSI ($r^2 = 0.97$), and the Kodak Ektachem ($r^2 = 0.91$). An analysis of Biosen compared with YSI revealed a positive bias of $0.37 \text{ mmol}\cdot\text{L}^{-1}$ (95% limits of agreement, -0.85 to $1.59 \text{ mmol}\cdot\text{L}^{-1}$). The test-retest reliability correlation was significant ($r^2 = 0.99$, $P < 0.05$), but a paired t -test revealed a small ($0.03 \text{ mmol}\cdot\text{L}^{-1}$, $P < 0.05$) significant difference. The coefficient of variation from the three investigators across the 20 samples ranged from 1.3 to 3%. Blood lactate concentration in resting blood samples did significantly increase in value ($0.2 \text{ mmol}\cdot\text{L}^{-1}$, $P < 0.05$) after 7-h exposure to the air, whereas there was no change in maximal exercise blood lactate values after 20-h exposure to the air. **Conclusions:** In a practical context, the Biosen 5030 lactate analyzer was comparable to the other analyzers giving fast reliable measures of blood lactate concentrations over the full range of values, which remained stable over extended periods at room temperature. **Key Words:** TEMPORAL RELIABILITY, RELIABILITY, VALIDITY, BLOOD LACTATE MEASUREMENT

The assessment of blood lactate concentrations is commonplace in exercise physiology laboratories, normally as a marker of the metabolic strain being experienced by the body (8). Measurement during exercise is frequently used as an indicator of performance ability and also to determine appropriate training intensities (2). With this increasing use of blood lactate concentrations there has also been a parallel increase in the number of analyzers designed to measure blood lactate concentrations. It is important that the validity and reliability of these analyzers is examined in the real laboratory study setting rather than accepting the manufacturer claims.

As reliability is a factor that greatly affects statistical power, many researchers are concerned about the reliability

of the exercise protocols they use to determine performance or threshold levels. Yet few investigators specifically measure (or at least quote) the reliability of the equipment they use to measure blood lactate. Those that have considered analyzer reliability have done so using a variety of methods, coefficient of variation, test-retest correlation, or standard errors, mean standard deviation. Although the most common, a simple correlation of test retest values that gives a measure of relative reliability is considered a poor method (1,3).

The method of blood collection, volume of blood required, stability of the sample, and cost and speed of measurement can restrict study design. Of the analyzers currently in use, many require the blood to be analyzed immediately or at best require an immediate procedure to mix the blood with an anticoagulant. In addition, the actual analysis process is relatively slow, normally taking at least 2 min to process each sample.

In terms of study design, faster lactate analyzers that provide rapid feedback during an exercise test would allow

protocol changes to occur during the test giving a greater degree of flexibility. Moreover, the use of smaller blood sample volumes that would remain stable for long periods without the need for cooling would enhance the ability to collect field data.

The Biosen 5030, a rapid whole blood lactate analyzer, has not been assessed for reliability and validity compared with other commonly used analyzers; therefore, the aims of this study were to: (i) evaluate the Biosen 5030 (EKF Industrie, Elektronik GmbH, Barleben, Germany) by comparing blood lactate values with a Yellow Springs Instruments 2300 (Yellow Springs, OH) lactate/glucose analyzer and a Kodak Ektachem DT II (Eastman Kodak, Rochester, NY) in a practical laboratory context; (ii) assess the reliability of the Biosen 5030 using a series of test-retest measurements; and (iii) assess the stability of samples (temporal reliability) left at room temperature for 7–20 h before analysis.

METHODS

As a part of other laboratory investigations, a series of maximal and submaximal exercise tests were performed to generate blood samples across a range of exercise intensities from rest to volitional exhaustion. The mode of exercise included both cycle ergometry (Kingcycle Rig, EDS Portaprompt, High Wycombe, UK) and treadmill running (Woodway XELG70, Weil am Rhein, Germany). The data generated was then used to: (i) assess the validity of the Biosen 5030 by comparing the values produced against those of the Yellow Springs Instruments 2300 lactate/glucose analyzer and a Kodak Ektachem DT II; (ii) assess the test-retest reliability of the Biosen 5030; (iii) determine the inter- and intra-investigator reliability when using the Biosen 5030; and (iv) assess the temporal reliability (stability) of samples containing both resting preexercise and maximal intensity exercise concentrations of lactate for analysis using the Biosen 5030.

Blood Sampling and Analysis

All blood samples used finger capillary blood following a puncture made using a lancet (Owen Mumford, Woodstock, UK). Blood samples for analysis using the Biosen 5030 were collected in 20- μ L capillary tubes and immediately mixed with a lysing stabilizing agent in a safe-lock vial. This involved shaking the sealed vial for approximately 15 s. Samples could then be analyzed immediately or left for a predesignated duration before analysis. Before blood analysis, the analyzer was calibrated with standard 12 mmol·L⁻¹ solution. Blood samples for analysis using the YSI 2300 were collected in a large capillary tube (50 μ L) and injected into a cuvette for immediate analysis (immediate analysis required to prevent clotting). Blood samples for analysis using the Kodak Ektachem DT II were collected in two large capillary tubes (50 μ L) and injected into a gel-cup (Statspin Technologies, Norwood, MA) before being centrifuged for

2 min at 12,000 rpm to separate the blood plasma for analysis.

Assessment of Validity

Triplicate blood samples (1 for each analyzer) were taken at various stages during a series of cycling performance tests. This generated 144 sets of triplicate blood samples for comparison between the three analyzers across a range of exercise intensities from resting to maximal exercise.

Test-Retest Reliability of the Instrument

During the tests described above in the section on the Assessment of Validity, additional blood samples were taken every minute for analysis using the Biosen 5030. This generated 665 blood samples covering a range of blood lactate values 0.85–16.37 mmol·L⁻¹. These were each measured twice using the Biosen 5030, with approximately 5 min between measurements. During this interval the sample was exposed to the atmosphere as a result of the puncture in the plastic vial made by the analyzer extracting the first sample.

Inter- and Intra-Investigator Reliability

To assess inter- and intra-reliability 20 resting capillarized blood samples were taken from three subjects (3–4 lancet punctures per subject, always in the same finger). Each subject was sampled by a different investigator. Intra-investigator reliability was assessed from the correlation coefficient (CV) of the 20 measurements; a comparison of these correlation coefficients was used to indicate intra-investigator reliability.

Temporal Reliability (Stability)

To measure the temporal stability of resting blood samples measured by the Biosen 5030, 20 replicate resting capillarized blood samples were taken from the finger of a subject. Ten of these samples were measured immediately. These samples now exposed to the air, and the 10 sealed unmeasured sealed samples were then analyzed after approximately 7 h stored at room temperature (18–22°C).

In addition to these resting samples, a further 36 duplicate samples were taken from subjects after exhaustive running or cycling exercise. One of the samples was measured immediately. This punctured vial was then stored with the second sealed sample at room temperature (18–22°C) for ~20 h before measurement. Comparisons between resting and maximal samples measured immediately and those measured after 7 or 20 h storage in a sealed vial would provide an indication of the temporal stability. The stability of sealed samples compared with those already measured (therefore, punctured and exposed to the atmosphere) permitted a quantification of the possible effect of oxidation occurring in these samples over time. The effect of lactate concentration on temporal stability was determined from the measurement of both the resting and maximal blood lactate concentrations stored for 7–20 h at room temperature.

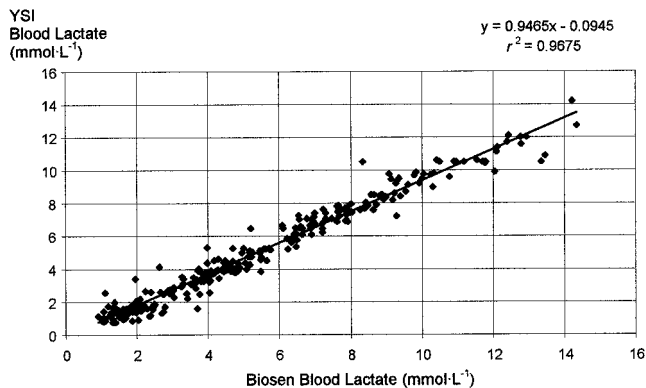


Figure 1—Correlation of Biosen versus YSI Blood Lactate ($\text{mmol}\cdot\text{L}^{-1}$) ($N = 276$).

Statistics

Values are reported as mean \pm standard deviation (SD). To assess the level of agreement between analyzers blood lactate values from the Biosen were correlated with those from the YSI and Kodak using a Pearson product moment correlation. In addition, a more precise statistical method of assessing the agreement between two measurement methods as described by Bland and Altman (3) was also applied to the Biosen-YSI comparison.

Reliability from the test-retest measurements was assessed using two-tailed paired Student *t*-tests, intra-class correlation (ICC) tests, and a Bland and Altman (3) analysis. Where Bland and Altman (3) analysis was used, data were checked for heteroscedasticity and a log transform was applied where appropriate as described by Atkinson and Nevill (1).

Temporal reliability (stability) was analyzed using two-way repeated measures ANOVA with appropriate Tukey *post hoc* analysis. Level of significance for all tests was set at $P < 0.05$.

RESULTS

Assessment of Validity

Biosen versus YSI. Figure 1 shows that there was a very strong significant relationship between the two analyzers with a coefficient of determination of 0.9675. The line of best fit, however, was not equivalent to the line of equality with the Biosen values being consistently higher across the range of values. There is no evidence of a greater disagreement between the measurements at any particular lactate value.

A more precise procedure for assessing the agreement between two methods of measurement is described by Bland and Altman (3). This method involves plotting the difference between the two measurements against the average of the two measurements (Fig. 2). This shows that there was an average bias of $0.37 \text{ mmol}\cdot\text{L}^{-1}$ and the 95% limits of agreement were 1.59 to $-0.85 \text{ mmol}\cdot\text{L}^{-1}$. Because of the large sample size, the standard error for these limits of agreement was very small ($0.06 \text{ mmol}\cdot\text{L}^{-1}$). Therefore, we

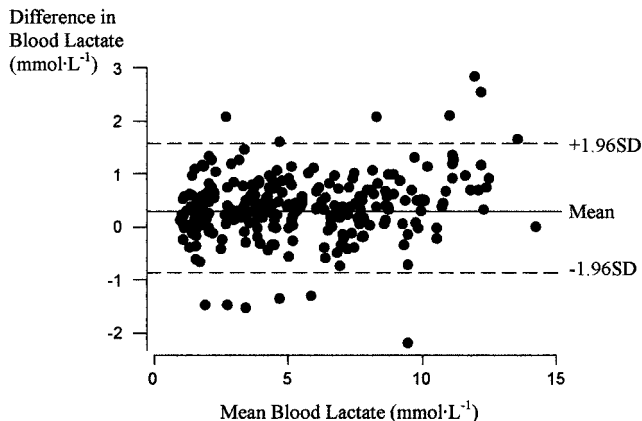


Figure 2—Bland and Altman (3) analysis for blood lactate data from the Biosen 5030 and the YSI 2300 ($\text{mmol}\cdot\text{L}^{-1}$).

can assume that these values are a representative estimate of the values that apply to the whole population.

Figure 2 also demonstrates that the bias is consistent across the range of lactate values and there is no evidence for a greater bias at the larger lactate concentrations. Confirmation of this can be obtained by plotting the absolute differences in lactate values from the two analyzers against the mean of the two values (1); this suggested that there is no evidence of heteroscedasticity

Biosen versus Kodak. When comparing the measured values from the Biosen and Kodak, it was not appropriate to use a Bland and Altman (3) analysis as the methods of analysis are based on different principles and do not measure exactly the same variable. The Kodak Ektachem DT II measures plasma lactate using a dry spectrophotometric method, whereas the Biosen measures whole blood lactate levels by using a wet chemistry method measuring the oxidation of lactate. On average, the Kodak measurements were $50.7 \pm 29\%$ higher than Biosen measures. Figure 3 illustrates that there was a significant correlation between the two values although this is somewhat more variable than the Biosen-YSI comparison as demonstrated by a lower coefficient of determination ($r^2 = 0.9061$).

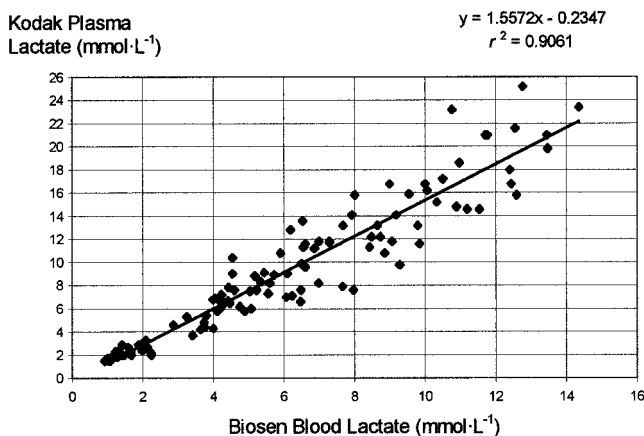


Figure 3—Correlation of Biosen blood lactate versus Kodak plasma lactate ($\text{mmol}\cdot\text{L}^{-1}$) ($N = 115$).

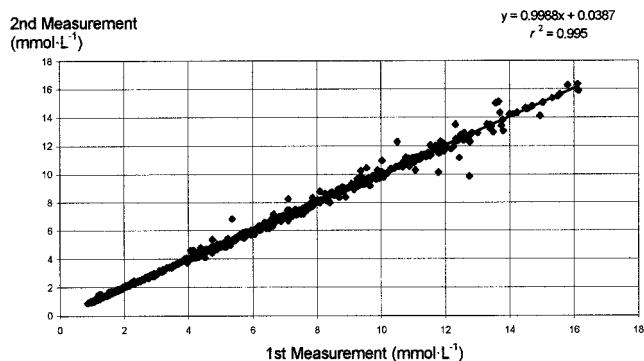


Figure 4—Biosen, test-retest of blood lactate ($\text{mmol}\cdot\text{L}^{-1}$) ($N = 665$).

Test-Retest Reliability of the Biosen

Figure 4 shows a significant correlation between the two measurements with measurements being particularly close to the line of best fit at the lower values and only a slight increase in variability at higher values. The strength of the relationship between the two measurements is indicated by the high coefficient of determination ($r^2 = 0.995$). However a paired *t*-test showed that the second measurement ($6.44 \pm 3.62 \text{ mmol}\cdot\text{L}^{-1}$) was significantly higher than the first ($6.41 \pm 3.61 \text{ mmol}\cdot\text{L}^{-1}$), the magnitude of this increase was very small ($0.03 \text{ mmol}\cdot\text{L}^{-1}$, 95% CI 0.01–0.05). A check of the test-retest data revealed that there was some evidence of heteroscedasticity therefore a Bland and Altman (3) analysis was carried out on the log transformed data. This showed that there was an average bias of 0.7% (95% limits of agreement of 7% below to 6% above). The calculated coefficient of variation of the test-retest data was 1.4%.

Inter- and Intra-Investigator Reliability

The calculated coefficients of variation with resting blood samples for the three investigators were 3, 1.3, and 1.3%, respectively.

Temporal Reliability

The three measurements of resting blood lactate concentrations analyzed using a two-way ANOVA indicated that there was no difference between the samples measured immediately, those remeasured after 7 h, or those that had been sealed before analyzing 7 h later. However, measurements of the samples that were reanalyzed after 7 h were significantly higher than those that had been stored for 7 h in a sealed vial. Even so, the magnitude of this difference was small ($0.223 \text{ mmol}\cdot\text{L}^{-1}$).

A comparison of the 36 duplicate maximal exercise samples by using a two-way ANOVA revealed that there was no difference in the blood lactate values for the samples measured immediately, remeasured after on average 18.8 h exposed to the atmosphere a room temperature, or measured after on average 18.8 h storage in sealed vials. (Table 1).

CONCLUSIONS

This study considered the use of a relatively new lactate analyzer. The Biosen 5030 blood lactate analyzer used in a practical laboratory study setting has been shown to give measured values very comparable to the widely used YSI 2300 lactate analyzer across a full range from resting to maximal exercise levels. This close correlation might be expected as both machines use similar electrode technology, L-lactate oxidase immobilized in a thin membrane placed over an electrochemical probe. The Bland and Altman (3) analysis did, however, reveal a small bias with the Biosen on average giving a measurement $0.37 \text{ mmol}\cdot\text{L}^{-1}$ higher than the YSI across the range of values, with no evidence of heteroscedasticity. Although, the magnitude of the bias is considered physiologically insignificant, in terms of normal exercise testing, and could reflect a bias in either machine.

The comparison with the Kodak Ektachem DT II did reveal more variable results, but this variability would seem to originate from the Kodak measurements. Although it should be highlighted that the Kodak method of analysis, dry, spectrophotometric, using blood plasma, could account for some of the differences. Previous research comparing plasma lactate with whole blood lactate concentrations have been inconsistent in their findings. Millard-Stafford et al. (9) found lactate values that were 91% and 97% higher values for the Kodak DT60 during rest and exercise, respectively, compared with the YSI. In contrast, Williams et al. (11) found plasma lactate values to be 48% higher than whole blood values, both measured on a YSI 23AM. This later finding agrees more closely with this study, which found that on average the Kodak measurements were 50.7% higher than Biosen measures.

Analysis of the test retest data revealed that there was evidence of heteroscedasticity, but the average bias of 0.7% would be considered small, although similar data are not available for other analyzers. However, the measured test retest coefficient of variation of 1.4% compares favorably with other studies using the YSI 2300 lactate analyzer, which quote CV of 0.8–3.3% within series and 1.2–3.7% within day (6,7,12).

From the various measures of reliability over a range of blood lactate concentrations taken by different investigators, the Biosen would seem to be very reliable with an overall coefficient of variation of less than 3% for resting blood lactate concentration. Considering the test-retest results, the

TABLE 1. Biosen, stability of resting and maximal blood samples over time ($\text{mmol}\cdot\text{L}^{-1}$).

	Measurement 1 ($\text{mmol}\cdot\text{L}^{-1}$)	Measurement 2 ($\text{mmol}\cdot\text{L}^{-1}$)	Measurement 3 ($\text{mmol}\cdot\text{L}^{-1}$)
Resting sample	0.811	0.955 ^a	0.732
Maximal sample	8.628	8.607	8.247

Measurement 1, batch 1 sampled immediately; measurement 2, batch 1 resampled after 7 h (resting) or 18.8 h (maximal); measurement 3, batch 2 sampled after 7 h (resting) or 18.8 h (maximal).

^aSignificantly different from measurement 3, resting sample ($P < 0.05$).

very small difference in the values are physiologically insignificant.

In addition, the resting and maximal exercise blood samples seem to be very stable for up to 20 h, whether stored at room temperature in a sealed vial or exposed to the air (as occurs in the analysis when the vial is punctured). For the resting samples exposed to the air, there was a slight increase in the recorded lactate values (Table 1), which appears to occur within 5 min but is relatively small and physiologically insignificant, although in contrast the maximal samples demonstrated no change in lactate concentration over much longer periods of time (~20 h).

This study has shown that compared with similar analyzers, the Biosen 5030 is a valid and reliable blood lactate analyzer over a range of blood lactate values. The good

temporal stability also enables samples to be taken in the field for later analysis in the laboratory. Furthermore, the rate of analysis is much faster than other equivalent analyzers, which coupled with an automatic sampling system provides the potential for the information on a subject's lactate levels to be available while a test is still in progress, which may be useful in new threshold tests like the lactate minimum test (4,5,10).

This study also provides a framework in terms of data collection and analysis for further assessment of other blood lactate analyzers.

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