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Evaluation of three portable lactate-measurement devices in exercising horses

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Summary: Since one of the validated handheld lactate (LA) measurement devices (Lactate Pro) is now out of production, an alternative instrument is to be found for equine exercise testing in the field and for use in horses with gastrointestinal disorders. The aim of this study was to evaluate three handheld LA analysers (Lactate Pro, Lactate Pro-2 and StatStrip Lactate Xpress), and to compare these with the gold standard laboratory assay (Lab-LA; DXC-600 Analyser - Beckman Coulterlab). A total of 215 blood samples were collected from the jugular vein of 44 eventing horses during standardized exercise testing. Immediately after collection each blood sample was parallel tested using the three lactate analysers. The rest of each blood sample was placed in NaF tubes and centrifuged within 8 hours. The plasma was stored at -20°C for laboratory assay. The results of each analyser were compared with the laboratory analyser. All data were statistically evaluated using a linear mixed effect model (Akaike's Information criterion; $t > 2.00$) and Partial Pearson correlations. The Partial Pearson correlations between Lab-LA and Lactate Pro, Lactate Pro-2 and StatStrip Lactate X-press were 0.974, 0.981, and 0.828 respectively. Of the three instruments the Lactate Pro-2 provided the closest correlation with the laboratory assay. The predicted values were derived from the formula: log (Lab-LA) = 0.27175 + 0.94567*(Lactate Pro-2) or Lab-LA = 1.3226*(Lactate Pro-2)0.94567. The predicted value for Lab-LA values using the Lactate Pro-2 was 0.992 for an average horse. The Lactate Pro-2 proved to be a good alternative for the Lactate Pro that is now no longer in production.

Keywords: exercise physiology, blood, equine, lactate-measurement device

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Introduction

The measurement of plasma lactate (LA) concentration is an essential part of the fitness evaluation of sport horses (*Hauss* et al. 2014, *Munsters* et al. 2014). LA measurements are also frequently used as a diagnostic and prognostic aid in the assessment of horses with colic. Additionally it can be useful for evaluation of the perfusion status in equine patients with systemic inflammatory response syndrome (SIRS) and in critically ill neonates (*Hauss* et al. 2014, *van den Boom* et al. 2010, *Henderson* 2013). Determination of LA concentrations is conventionally performed in laboratory analysers, using the concept of enzymatic analysis (*Butudom* et al. 2010). However, such analyses are expensive and inconvenient in field situations. Handheld instruments used for human LA are available and have been evaluated for their application in exercise testing of horses (*Munsters* et al. 2014). The Accusport™ and the Lactate Pro™ (Lac-Pro) analysers have proven to be accurate within limited ranges when used in equine exercise testing and in horses with colic (*van den Boom* et al.2010, *Delesalle* et al. 2007, *Evans* and *Golland* 1996, *Nieto* et al. 2015, *Sloet van Oldruitenborgh-Oosterbaan* et al. 2008a/b). *Kobayashi* (2007) showed that the Lactate Pro™ (Lac-Pro) instrument was more precise than the Accusport at low and moderately high LA concentrations (<10 mmol/L) (*Kobayasi* 2007). However, the Lac-Pro is no longer available and this was the reason for performing the present study. The aims of the study were to evaluate the Lac-Pro, Lactate Pro-2 (Lac-Pro2) and the StatStrip Lactate Xpress (Lac-Xpress) lactate analysers and to compare these with a standard laboratory assay (Lab-LA) to establish which instrument would be the most reliable and rapid, handheld instrument.

Material and Methods

The Animal Ethics Committee of Utrecht University concluded that the proposed study did not require ethical approval, as it did not qualify as an animal experiment under Dutch law. Individual horse owner's consent was obtained for all horses participating in this study.

Blood sampling

Venous blood samples were collected from 44 eventing horses during standardized exercise tests performed at the beginning of the competition season to assess their fitness level. Five blood samples were used from each horse since the exercise test consisted of a four-step incremental exercise test where blood samples were taken within 1 minute after finishing each step, and again after 10 minutes of recovery. The exercise test for the eventing horses is a standardised protocol authorized by the Dutch National Equestrian Federation (KNHS).

The exercise tests were carried out between January and March in 2014 and 2015. Some horses did not complete the entire exercise test. The test was stopped when horses rea-

ched a LA value >6 mmol/L before step 4. A total of 215 blood samples were collected: In 2014, 97 blood samples were taken from 20 horses and in 2015, 118 blood samples from 24 horses.

Immediately after the team veterinarian had collected a blood sample, three assistants, each holding one of the three portable LA-devices, (Lac-Pro and Lac-Pro2 and the Lac-Xpress) simultaneously analysed a drop of blood from the same sample. The rest of the blood was immediately stored in a sodium fluoride/potassium oxalate anticoagulant blood collection tube (Greiner Bio One, Alphen aan de Rijn, The Netheralnds) and stored at an ambient temperature of 2–9 °C (*Tennent-Brown* et al. 2010, *Henderson* 2013). All samples were centrifuged within 8 hours of arrival at the laboratory and then kept frozen (at -20 °C) until analysis could be performed in one batch using laboratory-based equipment (Lab-LA; DXC-600, Beckman Coulter).

Analysers

The Lactate Pro, Lactate Pro-2 from the manufacturer Arkay™ (Kyoto, Japan) and the StatStrip Lactate Xpress of Nova Biomedical (Waltham, USA), are portable blood LA analysers designed to measure LA in whole blood samples, collected from a fingertip or earlobe in human athletes. In table 1a summary of the characteristics of these devices is shown. In all measurement devices the plasma LA concentration was determined by the electrochemistry method. The laboratory analyser was maintained and calibrated according to the manufacturer's recommendation.

Statistics

The results from the Lac-Pro, Lac-Pro2, Lac-Xpress and Lab-LA were used to produce a normal probability plot to check the residuals for normality. If the normality assumption did not hold, the data were log transformed. Results were analysed using two different methods: the classic method with Partial Pearson Correlations, and a more novel method using the Akaike's Information Criterion (AIC). Plasma LA data were analysed using a logistic regression with Partial Pearson correlations between the gold standard laboratory assay and Lac-Pro, Lac-Pro2 and Lac-Xpress. P values < 0.05 were considered significant.

Plasma LA data were also analysed using a linear mixed effect model with horse as random effect and year, plasma LA values of Lac-Pro, Lac-Pro2 and Lac-Xpress as fixed effects. This was used as the starting model. The AIC was used to see if this model could be reduced, if no further reduction was possible this was used as the final model (*Burnham* and *Anderson* 2002). Effects in the final model, after reduction, were considered important. Results are presented in t-values as AIC was used; an effect size (t-value) of >2.00 (more than two times larger than the standard error) indicates a real difference. For

Table 1 Technical characteristics of the portable lactate analyzers according to the manufacturer's data.

Portable lactate analyser	Lactate Pro	Lactate Pro 2	StatStrip Lactate Xpress
Measures	Plasma LA	Plasma LA	Plasma LA
Measurement range	0.8-23.3 mmol/L	0.5 – 25.0 mmol/L	0.3 – 20.0 mmol/L
Test Methodology	Electrochemistry	Electrochemistry	Electrochemistry
Test strip volume	5 µl	0.3 µl	0.6 µl
Turnaround time	60 sec	15 sec	13 sec
Test ambient temperature	10 – 40 °C	5 – 40 °C	15 – 40 °C
Humidity	20 - 80 %	20 - 80 %	10 - 90 %

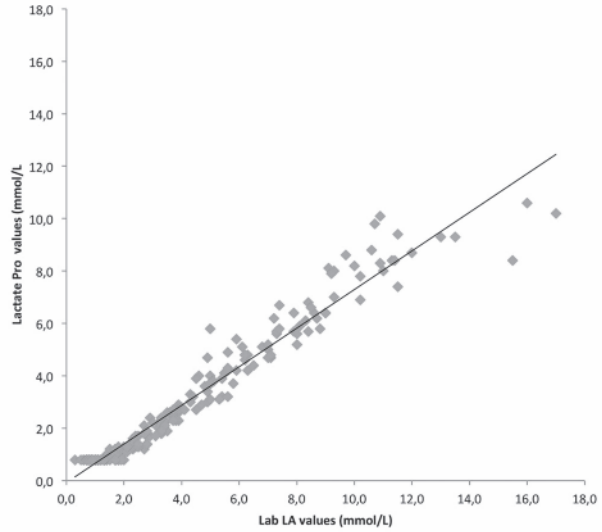

Fig. 1a Scatterplot of Lacate Pro LA values versus Laboratory assay values (Lab LA; golden standard) (n = 215)

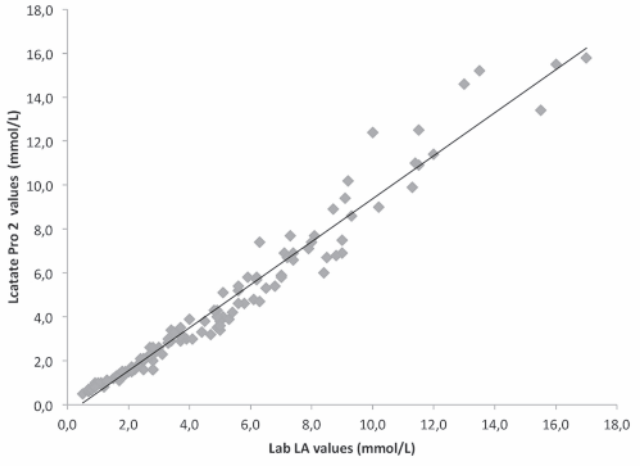

Fig. 1b Scatterplot of Lacate Pro 2 LA values versus Laboratory assay values (Lab LA; golden standard) (n = 129)

statistical analysis the software program (R:R Foundation for Statistical Computing, Vienna, Austria 2010) was used.

Results

General results

In 2014, all 97 blood samples were analysed with the Lab-LA, Lac-Pro and the Lac-Xpress and also 13 with the Lac-Pro2. In 2015, 118 blood samples were used to evaluate all three the portable devices compared to the Lab-LA. 215 blood samples were compared with the Lab-LA, Lac-Pro and Lac-Xpress and 129 blood samples with the Lab-LA and the Lac-Pro2. Lactate concentrations ranged from 0.8 mmol/L–17.0 mmol/L.

Results analysers

Partial Pearson correlations

The Partial Pearson correlations between the gold standard laboratory assay and Lac-Pro, Lac-Pro2 and Lac-Xpress were 0.976, 0.992 and 0.955 respectively (Figure 1a, 1b and 1c.

Linear mixed model

The correlation between the predicted values for model and the log(lab LA) values was 0.9945 for an average horse. The predicted values for an average horse and an 'average' year are given by: log (Lab-LA) −0.34275+0.13659*log(Lac-Xpress)+0.79507*log(Lac-Pro2). Both the Lac-Xpress and the Lac-Pro2 delivered better estimated values of lab-LA than the Lac-Pro (figures 1a, 1b, 1c). The correlation between Lac-Xpress and Lac-Pro2 was high (0.926). The instrument best predicting the Lab-LA was the Lac-Pro2, which showed the strongest relationship with Lab-LA according to the AIC. The Lac-Pro2 showed predicted values for the model of Lab-LA values of 0.9923 for an average horse. The predicted values for an average horse and an 'average' year are given by: log (Lab-LA) = 0.27175+0.94567* (Lac-Pro2) or Lab-LA=1.3226* (Lac-Pro2) 0.94567.

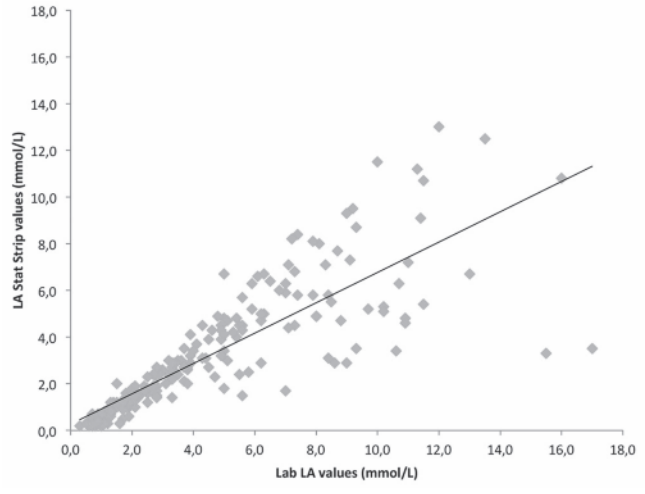

Fig. 1c Scatterplot of Lacate StatStrip (LA-Xpress) LA values versus Laboratory assay values (Lab LA; golden standard) (n=215)

Discussion

A handheld lactate analyser is useful in equine practice since the results are obtained more or less instantly and relevant clinical decisions can then be made immediately. In the present study, the Lac-Pro2 proved to be the most accurate device. The accuracy of the Lac-Pro was in accordance with previous studies (*Sloet van Oldruitenborgh-Oosterbaan* et al. 2008a). The Lac-Xpress had the lowest correlation compared to the gold standard showing both under- and overestimations of LA concentrations, especially in the higher ranges.

The Lac-Pro2 appeared to be a very practical analyser since the test strips were easy to handle and the result was delivered within 15 seconds. The latter is a very useful quality in equine exercise testing in the field as the exercise test can then be stopped immediately if the lactate concentration becomes too high. The Lac-Pro2 is also very useful in horses in colic cases since this instrument is also reliable at higher concentrations. The Lac-Pro was less reliable (*Nieto* et al. 2015, *Sloet van Oldruitenborgh-Oosterbaan* et al. 2008b).

In this study blood samples were analysed immediately using the handheld analysers. The samples were then stored for analysis in the laboratory as golden standard. *Tennent-Brown* et al. (2010) evaluated the stability of LA concentrations of blood stored in sodium fluoride/potassium oxalate tubes. Samples were refrigerated after collection and analysed 1, 3 and 6 hours after collection. There were no apparent changes in [LA] over time in stored samples compared to fresh blood samples.

All handheld LA devices proved to be most reliable at lower to moderate LA concentrations. Lac-Pro2 also demonstrated a good correlation with Lab-LA at moderate LA concentrations. In horses with gastro-intestinal disorders, plasma LA values above 8 mmol/L are indicative of a poor prognosis (*Delesalle* et al. 2007, *van den Boom* et al. 2010). Therefore, to assist in predicting survival in a colic horse Lac-Pro2 provides more reliable information within this range than the Lac-Pro. The latter device overestimated the LA values in this range (*Kobayasi* 2007). During exercise plasma LA concentrations can rise to 30 mmol/L (*Harris and Snow* 1988). These values are impossible to measure in the field since all the handheld devices used in this study have an upper limit of 20–25 mmol/L. Therefore, in exercise testing at high intensities a wet chemistry analyser will always be more reliable.

In conclusion: The Lac-Pro2 was the most accurate device in predicting laboratory plasma lactate concentration and will be a reliable substitute for the Lactate Pro which is no longer in production.

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Conflict of interest statement

This work has been supported finacially by the Dutch National Equestrian Federation (KNHS). Authors have declared no potential conflicts.

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