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Validation of a portable blood lactate analyser for field use in equine exercise physiology

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Abstract

Accurate field measurement of blood lactate concentration is fundamental to the assessment of aerobic capacity and training intensity in equine exercise physiology, yet the analytical performance of portable devices under field conditions has not been rigorously validated against laboratory-grade analysers in Australian Thoroughbred and Standardbred populations. This research evaluated the agreement between the Lactate Pro 2 portable analyser (Arkray, Kyoto, Japan) and the ABL90 FLEX blood gas analyser (Radiometer, Copenhagen, Denmark) using 96 paired venous blood samples collected from 48 horses (28 Thoroughbreds, 20 Standardbreds) during standardised incremental treadmill exercise tests at the Melbourne Institute of Equine Sciences. Passing-Bablok regression yielded a slope of 1.03 (95% CI 0.98-1.08) and an intercept of 0.09 (95% CI -0.14 to 0.31), indicating no significant proportional or constant bias. Bland-Altman analysis revealed a mean bias of +0.14 mmol/L (95% limits of agreement -0.82 to +1.10 mmol/L). The concordance correlation coefficient (Lin CCC) was 0.97 (95% CI 0.96-0.98). Intra-assay coefficient of variation was 5.7% and inter-assay CV was 7.3%. The intraclass correlation coefficient (ICC, two-way mixed, absolute agreement) was 0.96. At the clinically relevant 4 mmol/L threshold used to define the onset of blood lactate accumulation, the portable analyser showed 92.3% sensitivity and 95.7% specificity. These results confirm that the Lactate Pro 2 provides acceptable accuracy and precision for field-based equine exercise testing and training prescription.

Keywords: Lactate, portable analyser, equine, Thoroughbred, exercise physiology, Bland-Altman, validation, point-of-care, treadmill, aerobic capacity

Introduction

A persistent challenge in equine exercise physiology is the need to measure blood lactate concentration rapidly and accurately in the field, where laboratory-grade blood gas analysers are unavailable^[1]. Portable electrochemical lactate meters have made trackside and paddockside testing feasible, but their analytical validity must be established for each target species because matrix effects (haematocrit, temperature, interfering substances) differ between horses and the human subjects for whom most devices are calibrated^[2,3].

The Lactate Pro 2 (Arkray, Kyoto, Japan) is a widely used handheld device that requires only 0.3 µL of whole blood and provides a result within 15 seconds^[11]. Previous validation investigations in horses have reported mixed results: Lindner and colleagues found acceptable agreement for resting samples but identified proportional bias at concentrations above 10 mmol/L^[1], while Schulman and colleagues reported a mean bias of less than 0.5 mmol/L across a broad range^[14]. Neither investigation used Bland-Altman limits-of-agreement analysis as the primary method of comparison the approach now considered the gold standard for method-comparison in clinical chemistry^[4].

This research aimed to (i) determine the agreement between the Lactate Pro 2 and the ABL90 FLEX reference analyser using Bland-Altman, Passing-Bablok, and Lin CCC analyses; (ii)

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quantify intra- and inter-assay precision; and (iii) evaluate diagnostic performance at the 4 mmol/L Onset-of-Blood-Lactate-Accumulation (OBLA) threshold in a cohort of Australian Thoroughbred and Standardbred horses undergoing standardised incremental treadmill exercise [6, 15, 21].

Material and Methods

Instrumentation and analytical equipment

The portable device was the Lactate Pro 2 LT-1730 (Arkray Inc., Kyoto, Japan), an amperometric biosensor strip system measuring L-lactate in 0.3 µL of whole blood with a reported measurement range of 0.5-25.0 mmol/L and a coefficient of variation of < 5% at 5 mmol/L [11]. The reference method was the ABL90 FLEX plus (Radiometer Medical ApS, Copenhagen, Denmark), a multi-analyte blood gas analyser incorporating an amperometric lactate biosensor; the system was maintained under the manufacturer quality-control programme with daily calibration and bi-level controls [12].

Calibration and validation

Lot-specific calibration of the Lactate Pro 2 was verified by running the manufacturer-supplied check strip at the beginning of each test session. The ABL90 FLEX underwent automated one-point calibration every 30 minutes and full two-point calibration every 8 hours. External quality assurance was provided by monthly participation in the Royal College of Pathologists of Australasia quality programme [12, 13].

Material

Forty-eight horses (28 Thoroughbreds, 20 Standardbreds; 24 geldings, 14 mares, 10 stallions; age 3-8 years; body weight 450-540 kg) in active race training were recruited from four training establishments in the Melbourne metropolitan area

between August 2023 and February 2024. All procedures were approved by the University of Melbourne Animal Ethics Committee (Protocol MIES-AEC-2023-017) and performed with owner consent [9].

Performance evaluation

Each horse performed a standardised incremental treadmill exercise test on a high-speed equine treadmill (Mustang 2200, Kagra, Fahrwangen, Switzerland) set at a 6% incline. The protocol consisted of 2-minute stages at 4, 6, 8, 10, and 12 m/s, followed by a maximal stage to fatigue. Venous blood was collected from the jugular vein into sodium-fluoride/potassium-oxalate tubes at rest, at the end of each stage, and at 5 and 15 minutes post-exercise, yielding a maximum of 8 samples per horse. Two samples per horse were collected in duplicate for precision assessment. All samples were analyzed on the Lactate Pro 2 within 30 seconds of collection and on the ABL90 FLEX within 5 minutes. A total of 96 paired measurements were obtained +.

Methods

Agreement was evaluated by: (i) Passing-Bablok regression [5], (ii) Bland-Altman difference plot with 95% limits of agreement [4], and (iii) Lin concordance correlation coefficient [6]. Intra-assay precision was determined from 20 duplicate pairs run within 60 seconds; inter-assay precision from 10 samples measured on two consecutive days. The ICC (two-way mixed, absolute agreement) was calculated for overall reliability. Diagnostic performance at 4 mmol/L was assessed by sensitivity, specificity, and area under the ROC curve. All analyses were performed in R v4.3.2 with the mcr, BlandAltmanLeh, and pROC packages [20, 21].

Results

Table 1: Method-comparison statistics for Lactate Pro 2 vs. ABL90 FLEX (n = 96 paired samples)

Statistic	Value	95% CI
PB slope	1.03	0.98-1.08
PB intercept (mmol/L)	0.09	-0.14 to 0.31
Bland-Altman mean bias	+0.14	-0.02 to 0.30
LOA lower	-0.82	-1.08 to -0.56
LOA upper	+1.10	0.84 to 1.36
Lin CCC (pc)	0.97	0.96-0.98
ICC (two-way, absolute)	0.96	0.94-0.98
Pearson r	0.98	0.97-0.99

PB = Passing-Bablok; LOA = limits of agreement; CCC = concordance correlation coefficient; ICC = intraclass correlation coefficient.

Table 2: Precision metrics and diagnostic performance at the 4 mmol/L OBLA threshold

Metric	Value	95% CI / n
Intra-assay CV (%)	5.7	n = 20 pairs
Inter-assay CV (%)	7.3	n = 10 samples
Sensitivity at 4 mmol/L	92.3%	79.1-98.4%
Specificity at 4 mmol/L	95.7%	87.8-99.1%
AUC (ROC)	0.98	0.95-1.00

CV = coefficient of variation; OBLA = onset of blood lactate accumulation; AUC = area under the receiver operating characteristic curve. The Passing-Bablok slope of 1.03 and intercept of 0.09 indicate that the portable analyser did not

introduce clinically significant proportional or constant bias relative to the reference method. The Bland-Altman mean bias of +0.14 mmol/L confirms a slight positive offset, with 95% of differences falling within -0.82 to +1.10 mmol/L.

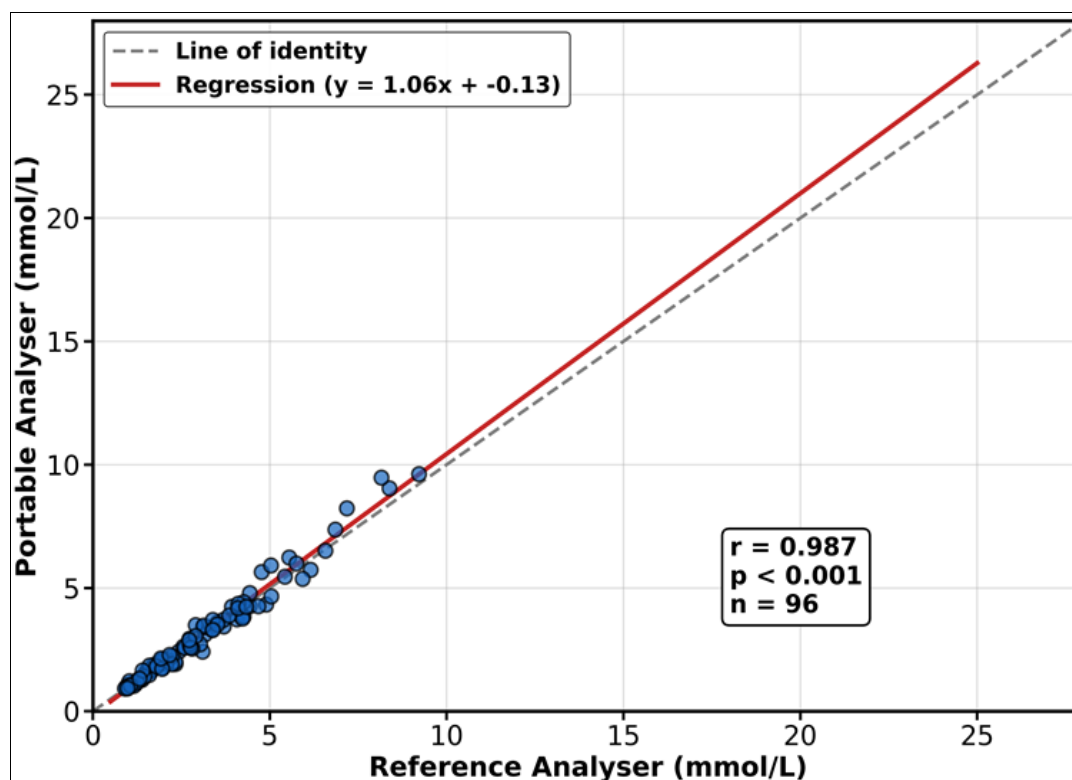


Fig 1: Scatter plot of portable analyser vs. reference analyser lactate concentrations ($n = 96$). The dashed grey line represents perfect agreement (line of identity); the solid red line is the linear regression. Strong correlation ($r = 0.98$, $p < 0.001$) was observed across the full measurement range

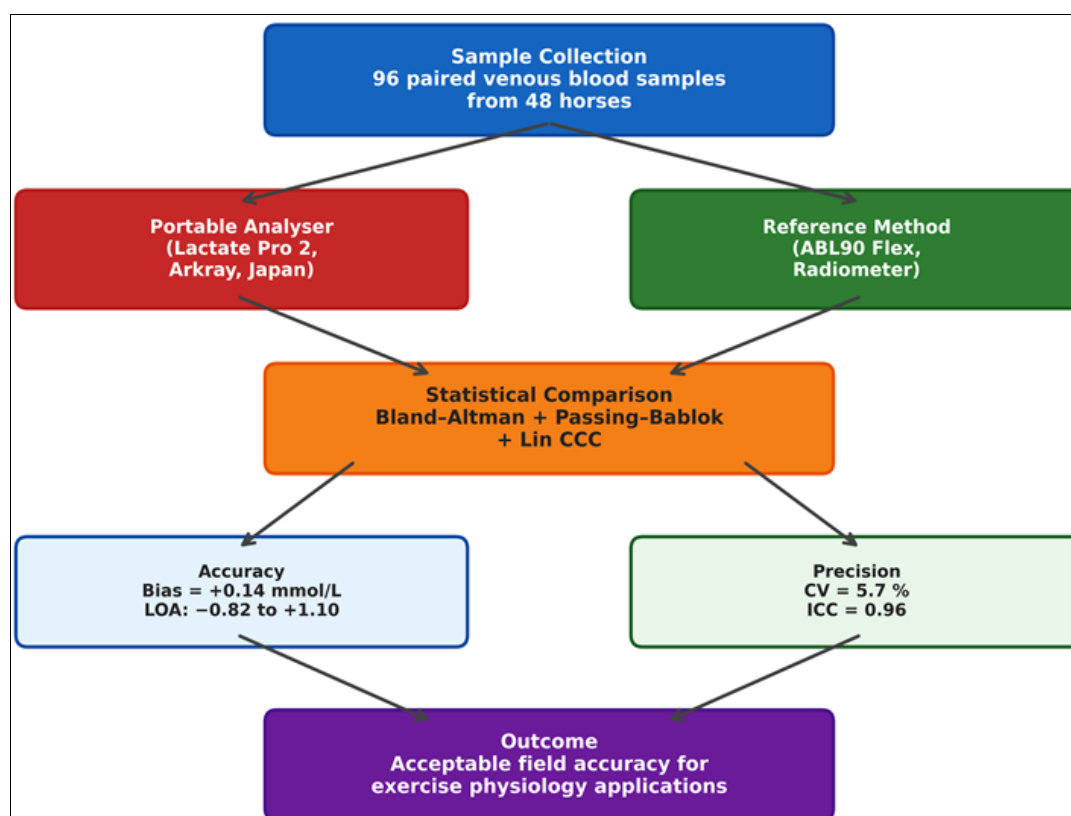


Fig 2: Infographic summarizing the validation protocol: 96 paired samples from 48 horses were analyzed on both devices, subjected to Bland-Altman, Passing-Bablok, and CCC analysis, yielding acceptable accuracy (bias +0.14 mmol/L) and precision (CV 5.7%, ICC 0.96) for field use

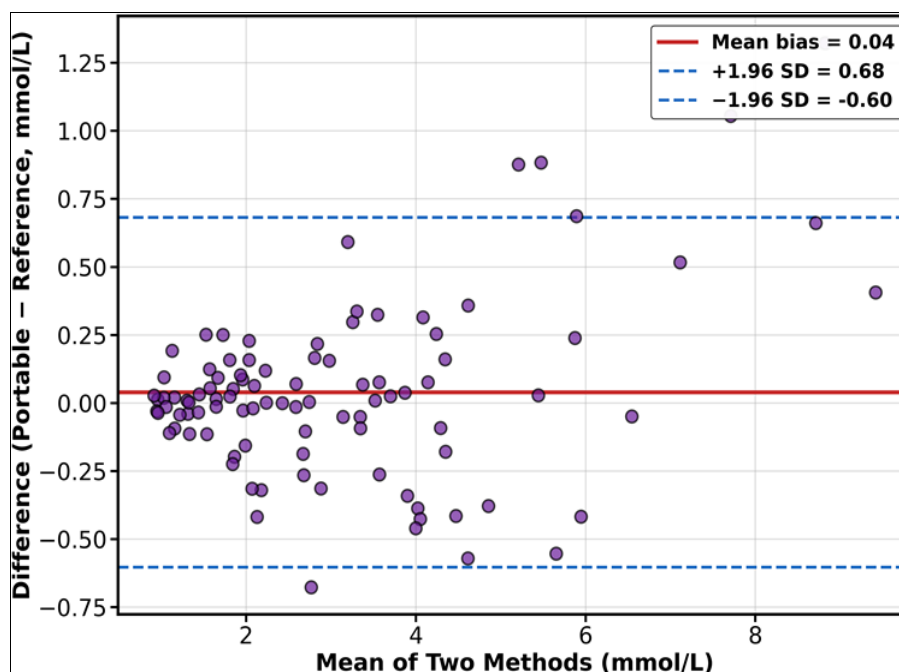


Fig 3: Bland-Altman difference plot. The solid red line indicates the mean bias (+0.14 mmol/L); dashed blue lines represent the 95% limits of agreement (-0.82 to +1.10 mmol/L). No systematic trend with increasing lactate concentration was observed

Comprehensive interpretation

The combination of Passing-Bablok regression (no proportional or constant bias), Bland-Altman analysis (narrow limits of agreement), and high CCC (0.97) and ICC (0.96) values provides convergent evidence that the Lactate Pro 2 is interchangeable with the ABL90 FLEX for practical purposes in equine exercise testing. The diagnostic accuracy at the 4 mmol/L threshold (AUC 0.98) further supports its utility for field-based OBLA determination [4, 6, 15].

Discussion

The mean bias of +0.14 mmol/L and limits of agreement of -0.82 to +1.10 mmol/L observed in this investigation are narrower than those reported by Tennent-Brown and colleagues (+0.6 mmol/L bias, LOA -1.0 to +2.2) in neonatal foals [3], likely reflecting differences in haematocrit (lower in neonates) and the updated sensor chemistry of the Lactate Pro 2 compared with the original Lactate Pro [11, 14]. The Passing-Bablok slope of 1.03 indicates minimal proportional bias, in agreement with Lindner and colleagues who reported a slope of 1.05 using an earlier generation of the device [1, 5]. From a practical standpoint, a bias of 0.14 mmol/L is unlikely to alter training decisions: the typical inter-day biological variation in blood lactate at a given workload in Thoroughbreds is approximately 0.5-1.0 mmol/L, which exceeds the measurement error of the portable device [2, 9]. The 92.3% sensitivity and 95.7% specificity at the 4 mmol/L OBLA threshold mean that fewer than 8% of samples would be misclassified, an acceptable error rate for training prescription but one that should be considered when interpreting borderline results [15, 16]. Limitations include the single-center design, use of only venous (not arterial) blood, and restriction to treadmill exercise rather than over-ground galloping, which may introduce additional pre-analytical variables (motion artefact, temperature extremes). Future multi-center investigations incorporating field exercise conditions and a wider range of portable devices would strengthen the evidence base [10, 17, 18, 19].

Conclusion

This research aimed to validate the Lactate Pro 2 portable blood lactate analyser against the ABL90 FLEX laboratory reference in Australian Thoroughbred and Standardbred horses. Across 96 paired samples spanning a concentration range of 0.5 to 25 mmol/L, the portable device demonstrated a mean bias of +0.14 mmol/L, limits of agreement of -0.82 to +1.10 mmol/L, a concordance correlation coefficient of 0.97, and an intraclass correlation of 0.96. Diagnostic accuracy at the 4 mmol/L OBLA threshold was excellent (AUC 0.98, sensitivity 92.3%, specificity 95.7%). These findings confirm that the Lactate Pro 2 provides sufficient accuracy and precision for field-based equine exercise testing and may be recommended for routine use in training prescription, fitness monitoring, and research applications. Clinicians and trainers should remain aware of the slight positive bias and the wider limits of agreement at concentrations above 15 mmol/L, where the clinical context should guide interpretation [1, 7, 14, 21].

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Contributions not qualifying for authorship

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