

Use of the Accusport semi-automated analyser to determine blood lactate as an aid in the clinical assessment of horses with colic

M L Schulman^a, J P Nurton^b and A J Guthrie^b

ABSTRACT

The most useful diagnostic methods in the initial evaluation of horses with colic assess the morphological and functional status of the gastrointestinal tract and cardiovascular status. This evaluation is best achieved using a combination of clinical and laboratory data. Blood lactate concentration (BL) is one of these variables. BL rises mainly due to poor tissue perfusion and anaerobic glycolysis associated with shock, providing an indicator of both the severity of disease and its prognosis. A hand-held lactate meter, Accusport, provides a rapid (60 seconds), inexpensive dry-chemical-based determination of BL. This trial evaluated the Accusport's ability to provide BL data as an adjunct to the initial clinical evaluation of horses with colic. The accuracy of the Accusport was tested by evaluation of its interchangeability with the benchmark enzymatic kit evaluation of BL in a trial using data collected firstly from 10 clinically normal control horses and subsequently from 48 horses presented with signs of colic. The BL values were recorded together with the clinical variables of heart rate (HR), capillary refill time (CRT), haematocrit (Hct), and pain character and severity on the initial assessment of the colic horses. Information regarding choice of therapeutic management (medical or surgical) and eventual case outcome (full recovery or died/euthanased) was recorded. The Accusport was found to be interchangeable with the enzymatic kit for recording BL values in colic horses with BL <10 mmol/l, which is within the BL range associated with survival. The interchangeability of an additional, laboratory-based wet chemical assay for BL, the Stat 7 was simultaneously evaluated for the colic and control horses. The Stat 7 was found to be interchangeable with the enzymatic kit for BL determination of colic horses. No linear associations between BL values with HR, CRT, Hct or pain assessment were observed. No relationship with either selection of therapeutic method or eventual case outcome was observed. All horses with BL >8 mmol/l died or were euthanased.

Key words: Accusport, blood lactate, colic, horse.

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INTRODUCTION

Diagnostic methods that evaluate the morphological and functional status of the gastrointestinal tract and the animal's cardiovascular status are most useful in the initial assessment of equine colic cases¹. A lethal outcome in a colic case is primarily a result of acute circulatory failure and shock following intestinal ischaemia. Blood lactate concentration (BL) increases during shock mainly due to poor tissue perfusion and anaerobic glycolysis^{4,8,10,11}. While BL provides a sensitive biochemical indicator of peripheral tissue perfusion (reflecting both the

severity of disease and its prognosis)^{8,10}, the best prognostic predictors of altered cardiovascular status are: degree of mental depression, heart rate (HR), oral mucosal colour, capillary refill time (CRT), jugular filling rate, arterial blood pressure, packed cell volume, anion gap and blood urea nitrogen concentration^{10–13,16}.

In general, field cases of colic are successfully managed medically. A smaller percentage (reportedly <10 % of cases) require swift surgical intervention to correct an obstructive lesion³. Clinical signs reflect the severity of disease in most cases. The diagnostic and prognostic value of any clinical finding is, however, dependent on the examiner's skills, experience and interpretation³. Appropriate and objective clinicopathological data can be used to enhance the objectivity of the prognosis and diagnosis^{8,10,11}. Evaluation

of clinicopathological data necessitates access to laboratory facilities, which is subject to practical constraints. There are often limitations with respect to both the availability and economics of the various laboratory tests. Colic cases by their nature and aetiologies require rapid evaluation, prognosis and therapeutic management³. This is particularly relevant in identifying potentially successful surgical cases. A hand-held lactate meter, Accusport (Boehringer Mannheim GmbH) has recently become available and provides a rapid, inexpensive dry-chemical-based determination of BL concentration within 60 seconds. The lactate concentration is determined from plasma by photometry after whole blood placed on a dry chemistry strip enters a measuring chamber.

The Accusport was designed to measure BL in human athletes, but several recent studies have evaluated its application in BL determination of equine athletes^{4,5,7,16}. The concentration of whole blood lactate is calculated by a conversion formula valid for humans. These conversion formulae appear to be applicable to the horse^{7,16}. In horses, unlike humans, the correlation between plasma and whole blood lactate is narrow, with increasing plasma to whole blood ratio as blood lactate rises. The ratio change is too small to markedly affect blood lactate as determined by either wet or dry chemistry methods. While a number of studies have discussed the value of BL determination in equine colic cases, no studies have reported the use of the Accusport in the assessment of the colic horse. The instrument's potential clinical use is dependent on the provision of accurate data in the determination of BL within the expected range observed in horses with colic.

This trial evaluated the Accusport's ability to determine BL as an adjunct to the clinical assessment of equine colic cases. Determinations of BL by the Accusport were compared with an enzymatic method⁹. In addition, these data were compared with those determined using a Nova Stat Profile Plus 7L semi-automated analyser (NOVA Biomedical).

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MATERIALS AND METHODS

Data for the evaluation of the Accusport were collected in 2 phases. The first phase was to determine a normal range of BL and clinical data from clinically healthy horses. During the second part of the study, BL and clinical data were determined in horses presenting with signs consistent with colic of various aetiologies.

Controls

Baseline data were collected from 10 clinically healthy Thoroughbred horses resident at the Equine Research Centre, Onderstepoort. None of these horses had shown clinical signs of colic during the previous 14 days. These horses underwent a clinical examination using the protocol for initial case assessment at the Equine Clinic, Veterinary Academic Hospital, Onderstepoort. This examination included assessment of HR, CRT, haematocrit (Hct), pain character and severity. In addition, 10 ml of venous blood was collected from each horse in a Vacutainer® tube containing lithium heparin. This tube was stored at 4 °C for not more than 2 hours before the lactate concentration was determined. The lactate concentration of each blood sample was determined using:

- 1) a semi-automated analyser (Nova Stat Profile Plus 7 L),
- 2) the Accusport analyser, and
- 3) spectrophotometrically using an enzymatic assay kit (Lact MPR3).

The collected Vacutainer® tube was inverted several times manually before analysis by the Nova Stat Profile Plus 7 L (Stat 7). The rubber seal of the Vacutainer® tube was removed to allow aspiration of the required specimen by the Stat 7 probe. The instrument performed the analysis and printed a result. A single replicate was recorded for each sample.

With the Accusport analyser, a drop of whole blood was placed on the test pad on a pre-blanked test strip, which was then placed in the Accusport analyser. The concentration was displayed after 60 seconds. Three successive readings were recorded for each sample and a mean of these was calculated. The same operator recorded all readings.

The remainder of each sample was then centrifuged at 2000 rpm for 10 minutes. The plasma was harvested and stored at -20 °C. Following the collection of all samples, the lactate concentration of each was determined spectrophotometrically (DU 650, Beckman Instruments), using an enzymatic assay kit, Lact MPR3 (Boehringer Mannheim GmbH). A single replicate was recorded.

Colic cases

In the second part of the study, data were collected from 48 horses presented to the Equine Clinic, Veterinary Academic Hospital. All horses presented with clinical signs consistent with colic due to a variety of causes. The standard protocol for initial case assessment was completed on arrival and 10 ml of venous blood was collected in a Vacutainer® tube containing lithium heparin. This tube was stored at 4 °C for not more than 2 hours, following which lactate concentrations were determined using the 3 methods described above. Information regarding the therapeutic management and outcome of the individual colic cases was obtained from the hospital records.

Statistical analysis

The method described by Lee *et al.*⁶ for assessing whether or not 2 different methods for measuring a specific quantitative outcome yield similar results (and are thus interchangeable) was used in this study. The correct statistic for measuring

the interchangeability (or the extent of agreement) between methods is the *intraclass correlation (r1)*. If $r = 0$, agreement between the 2 methods was based solely on chance. A positive $r1$ or a negative $r1$ reflects agreement greater than, or less than that based on chance alone, respectively. Meaningful agreement is reached when the population interclass correlation (lower limit of the 95 % confidence interval for $r1$) is ≥ 0.75 . Secondly, the pattern of concordance or discord should also be visually assessed. Thirdly, the 2 methods must not be shown to depict marked additive or non-additive systematic bias. Finally, the difference between the means of the 2 methods must not be significantly different from 0. Significance was set at $P < 0.05$.

RESULTS

Table 1 summarises the results of BL of control ($n = 10$) and colic horses ($n = 48$) as determined by each of the 3 methods. The statistics summarising the extent of agreement between the methods are

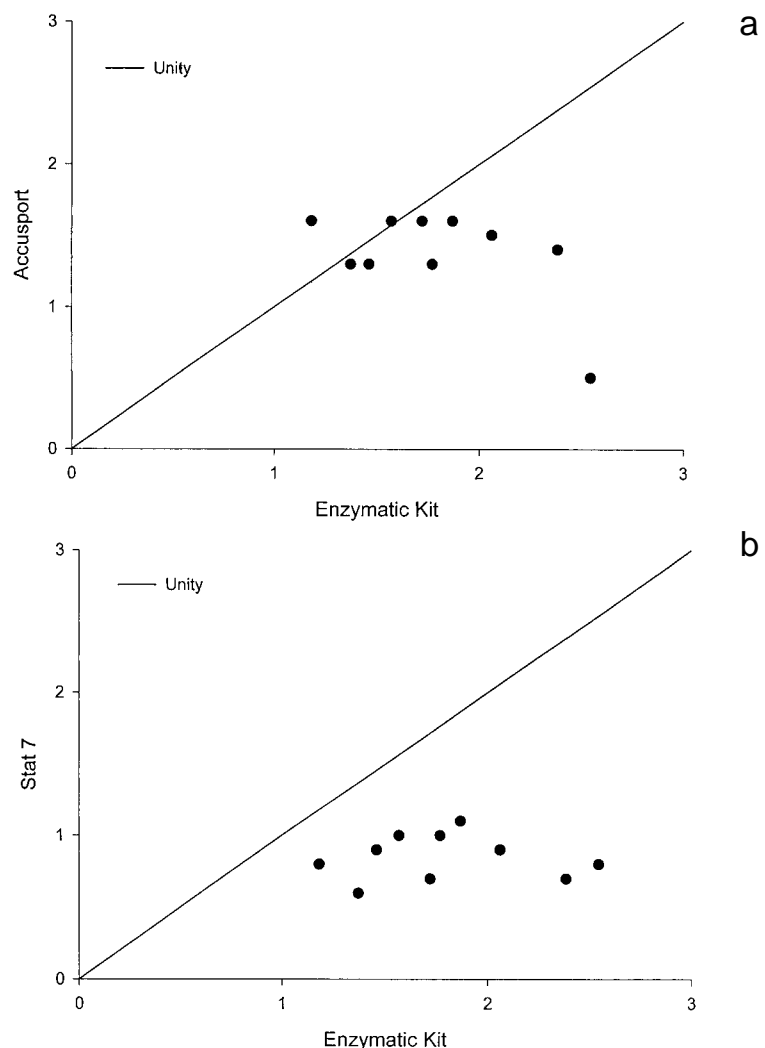


Fig. 1: Graphic representation of the extent of agreement of blood lactate measurements of control horses ($n = 10$) using the 2 methods, a) enzymatic kit vs Accusport, and b) enzymatic kit vs Stat 7.

Table 1: Descriptive statistics for blood lactate (mmol/l) of control ($n = 10$) and colic horses ($n = 48$) recorded by each of 3 different methods.

	Method of determination	Median	Min-max	Mean	SD
Control ($n = 10$)	Enzymatic kit	1.75	1.18–2.54	1.79	0.44
	Accusport	1.45	0.50–1.60	1.37	0.33
	Stat 7	0.85	0.60–1.10	0.85	0.16
Colic ($n = 48$)	Enzymatic kit	2.90	0.80–19.30	4.87	4.64
	Accusport	3.15	1.10–13.20	3.80	2.76
	Stat 7	2.60	0.40–19.60	4.69	4.91

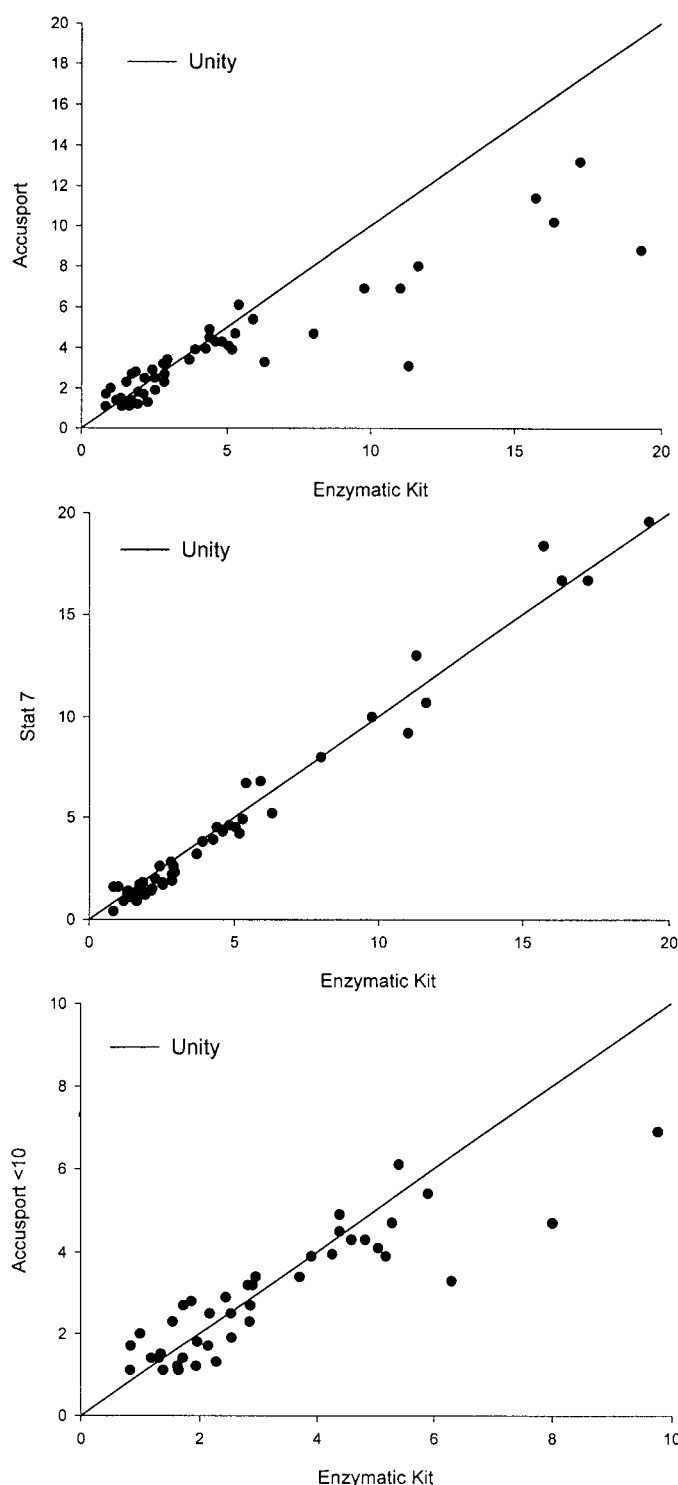


Fig. 2: Graphic representation of the extent of agreement of blood lactate measurements of colic horses using the 2 methods, a) enzymatic kit vs Accusport ($n = 48$), b) enzymatic kit vs Stat ($n = 48$), and c) enzymatic kit vs Accusport <10 mmol/l ($n = 41$).

shown in Table 2. Figures 1 and 2 provide a graphic representation of the extent of agreement between data obtained using the different methods in control and colic horses, respectively.

In the control horses ($n = 10$), the lower limit of the 95 % CI of $r1$ was <0.75 for the comparison of the data obtained by both the Accusport and the Stat 7 with the enzymatic kit. Secondly, comparison of the results obtained using the 2 methods show a wide difference from unity (see Fig. 1). Furthermore, the difference between the means (–0.94) for data obtained on the Stat 7 and the enzymatic kit was significantly different from 0. This confirms that neither Accusport nor the Stat 7 methods were interchangeable with the enzymatic kit in the control horses.

In the colic horses ($n = 48$), the lower limit of the 95 % CI of $r1$ was <0.75 for the comparison of the data obtained by the Accusport with the enzymatic kit. Comparison of the results obtained using the 2 methods show a deviation from unity (see Fig. 2a). Furthermore, the difference between the means obtained using the Accusport and the enzymatic kit were significantly different from 0. They are thus not interchangeable. In the case of the comparison between the Stat 7 and the enzymatic kit, the 95 % CI was >0.75. The means difference was not significantly different from 0. The comparison between Stat 7 and the enzymatic kit approached unity (Fig. 2b), thus the 2 methods are interchangeable.

In the colic horses in which the values measured by the Accusport were <10 mmol/l, the 95 % CI of $r1$ was 0.75. The comparison approached unity (Fig. 2c). The mean difference was not significantly different from 0. This indicates that the 2 methods were interchangeable for colic horses with a BL <10 mmol/l.

The second aspect investigated in this study was a series of comparisons between BL as measured on the Accusport and a number of selected clinical and haematological variables. All recorded values were obtained on initial evaluation of 48 horses referred with signs consistent with colic

Table 2: Summary of statistics of the extent of agreement for both control and colic horses of blood lactate as measured by the 3 determination methods.

	Method of determination	Difference between means	95 % CI	Interchangeability
Control horses (n = 10)	Enzymatic Kit vs Accusport	-0.42	-0.64	No
	Enzymatic Kit vs Stat 7	-0.94 ^c	-0.07	No
Colic cases	Enzymatic Kit vs Accusport ^a	-1.08 ^c	0.63	No
	Enzymatic Kit vs Stat 7 ^a	-0.19	0.98	Yes
	Enzymatic Kit vs Accusport ^b	-0.26	0.75	Yes

^aBlood lactate values for all colic cases (n = 48).

^bBlood lactate values for all colic cases with blood lactate <10 mmol/l (n = 41).

^cSignificantly different from 0.

of various causes. Comparisons were also made between BL and, firstly, choice of subsequent treatment (medical or surgical) and, secondly, with case outcome (full recovery or died/euthanased).

Figure 3 shows the comparison of BL with the 3 clinical variables of HR, Hct and CRT. The comparison of HR with BL fails to display a linear trend, which is particularly evident at the higher recorded values for the 2 variables (Fig. 3a). No linear relationship was seen between the variables of BL and Hct (Fig. 3b). The relationship between BL and CRT shows no linear trend between the recorded values (Fig 3c). Fig. 4 shows the relationship between BL and the categories of clinically determined pain. 'Depression' is associated with a broad range of BL from <2 to >10 mmol/l. The category of 'uncontrollable, continuous' pain is in several cases associated with a level of <2 mmol/l. There is obviously a poor relationship between both the severity and type of pain, with the BL recorded in an individual colic horse.

The chosen treatment method (medical or surgical) is shown not to be related to the BL on initial presentation (Fig. 5a). The BL as compared with the case outcome (died/euthanased or full recovery)

is shown (Fig. 5b). There was no linear trend or relationship. All colic cases (n = 5) with a BL of >8 mmol/l were associated with a lethal outcome.

DISCUSSION

Interchangeability with the accepted benchmark method for measurement of BL was used to evaluate the Accusport and the Stat 7. The choice of interchangeability imposes stringent criteria. These criteria are not entirely fulfilled by the Accusport across the entire range for either the clinically normal control horses (n = 10) or the colic horses (n = 48) sampled in this trial. These criteria were met by the Stat 7 when considering the colic horses, but not for the control horses. These criteria were, however, met by the Accusport when considering colic horses with Accusport measurements of BL <10 mmol/l (n = 41). It is generally accepted that few or no horses survive beyond this threshold value.^{2,10,12,13} This finding, although measuring BL and for a narrower range (<10 mmol/l), also supports the study of Evans and Golland⁴, who obtained accurate plasma lactate measurements in the range of 0.8–20 mmol/l, and Simmons *et al.*¹⁵ who observed that the Accusport was reliable in measuring

plasma lactates <13 mmol/l. This suggests that the Accusport is an acceptable method for evaluating horses within the range associated with a prognosis for survival.

The data showed that both the Stat 7 and Accusport consistently measured lower BL values than the enzymatic kit method. This is consistent with the findings of Lindner⁷ and Evans and Golland⁴. The latter also showed an underestimation of blood lactate concentration at a PCV > 50 %. The simultaneous rise in PCV in the drop of whole blood (as seen in some horses with pain and dehydration) is postulated to result in a decreased volume of plasma being available to seep through the dry chemistry strip. Alternatively, Accusport may measure a lower plasma lactate concentration due to increased osmotic fragility of erythrocytes. This may be caused by increased lysis on the dry chemistry strip releasing a greater volume of water to dilute the plasma lactate concentration. This reported effect of PCV on the recorded blood lactate values may require additional measures in those cases with a PCV > 50 % as well as for BL in the higher range. Thus the measurement of blood lactate may need repeating, or an alternative method,

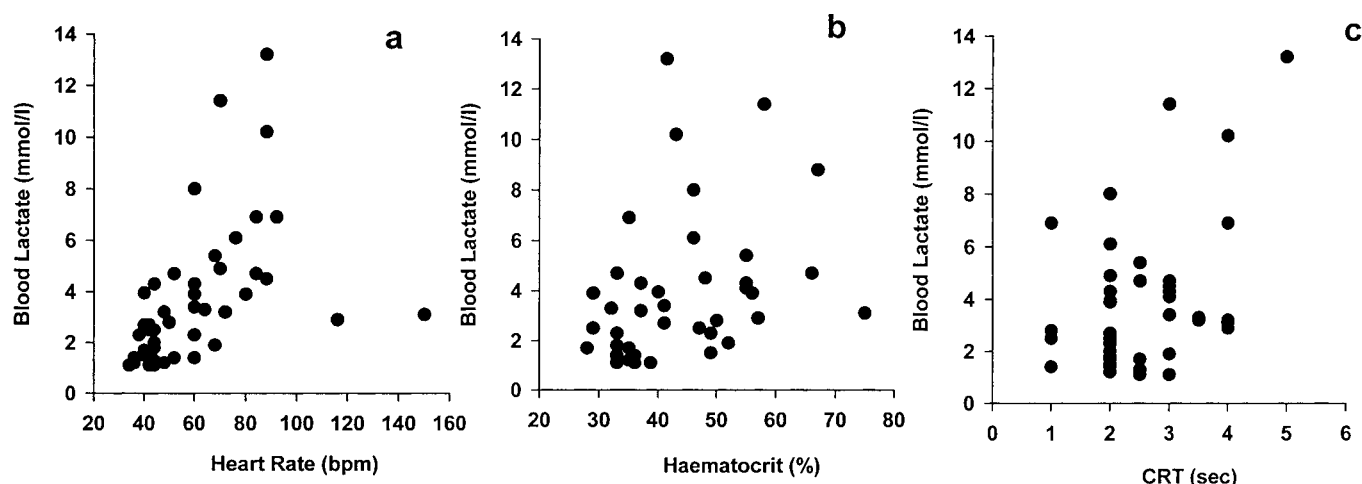


Fig. 3: Comparison of blood lactate measurements using Accusport with 3 clinical variables: a) blood lactate vs heart rate (HR), b) blood lactate vs haematocrit (Hct), and c) blood lactate vs capillary refill time (CRT).

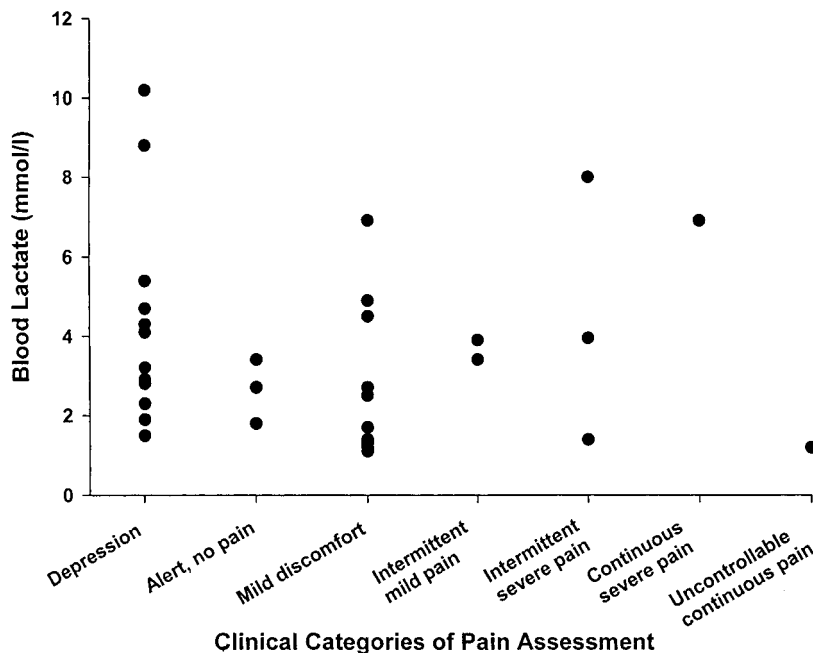


Fig. 4: Comparison of blood lactate measurements, using Accusport with clinically determined categories of pain assessment.

such as wet chemistry, chosen. The serial dilution of plasma samples may overcome this problem¹⁵, particularly for BL at higher concentrations.

This study also evaluated selected variables integral to the widely-accepted protocol for initial evaluation of colic horses. These variables evaluate cardio-

vascular status and are associated with accurate prognosis for survival. Although a clinician's skills, experience and interpretation play a role, each variable is readily and reasonably objectively evaluated. The Accusport can potentially fulfill the requirements of providing inexpensive and rapidly available clinicopatho-

logical data. This combined with clinical assessment is superior to relying on any isolated variable to provide rapid and accurate decisions in colic management.

No relationship was established between the BL at initial assessment with the choice of therapeutic option. This supports other studies where BL was seen to be a poor criterion for therapeutic choice¹⁰. This option was obviously influenced by several additional factors. These may include the clinician's experience, presumptive diagnosis, the horse's value and additional clinical or laboratory-derived data. These alone or in combination may prove more useful in the decision of how to optimally treat the individual horse.

No linear relationship was recorded for the relationship with the eventual case outcome as recorded in the Veterinary Academic Hospital records. Records are available up to the date of discharge of the individual horse, thus constituting a short- to medium-term outcome. The most relevant information derived from this aspect of the study was the observation that no horse with an initial BL >8 mmol/l survived. This is consistent with other studies^{2,10,12,13}. This also serves as a retrospective justification for electing in this study to eliminate all data with BL of >10 mmol/l in establishing the interchangeability of Accusport with the kit method of BL determination in colic cases.

The 'non-recovery' outcome category is influenced by several non-clinical factors. Economic considerations not only influence the selection of therapeutic option, but also additionally affect the decision to euthanase. This may relate directly to the indications for surgical intervention, or to satisfactory 'case outcome' as a whole. The probable return to full usage also influences the decision-making around euthanasia.

This study must be seen to support other studies^{3,8,11,14}. These suggest that no individual variable can be used in isolation to provide an accurate assessment of a case to allow reliable decision-making with respect to either the prognosis for survival or therapeutic option. It is probably relevant to suggest that for most colic cases the Accusport will prove adequate for the purpose of establishing BL consistent with inclusion in an initial assessment.

No prognostic method is infallible in distinguishing between survival and death. The survival rates and hence success of surgical cases in turn can be expected to improve if firstly, a hopeless prognosis is eliminated and secondly, earlier recognition of the need for surgical intervention is achieved.

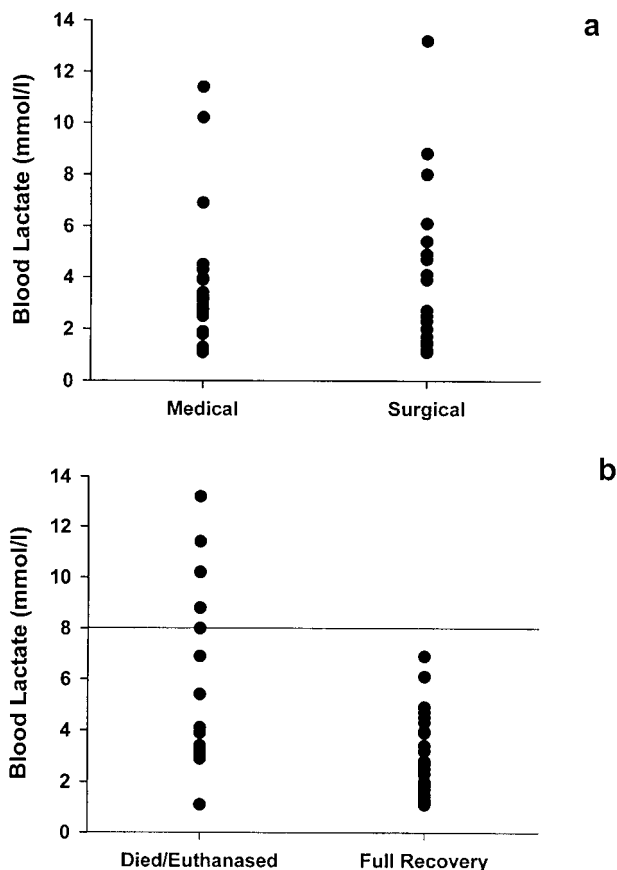


Fig. 5: Comparison of blood lactate measurements, using Accusport with a) the chosen method of treatment and b) the outcome of the case.

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