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Author/s:

Rosenstein, Patricia

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Clinical use of plasma lactate concentration and evaluation of the analytical performance of two benchtop analyzers for the measurement of L-lactate in canine plasma

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2018

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**CLINICAL USE OF PLASMA LACTATE CONCENTRATION AND EVALUATION OF THE  
ANALYTICAL PERFORMANCE OF TWO BENCHTOP ANALYZERS FOR THE MEASUREMENT  
OF L-LACTATE IN CANINE PLASMA**

BY PATRICIA ROSENSTEIN  
ORCID ID: 0000-0002-8824-7125

Submitted in partial fulfillment of the Master of Veterinary Science degree in the Faculty of  
Veterinary and Agricultural Science of the University of Melbourne

Submitted: July 2018

## ABSTRACT

Lactate has been used in small animal veterinary medicine since the 1990's. Ongoing reviews of the literature had revealed that an up to date, comprehensive review of the use of lactate in small animal clinical practice was overdue. Consequently, this became the first objective of this thesis (Chapters 1 and 2). Through this review process, a surprising gap was identified in the veterinary literature. Despite widespread integration into clinical practice and validation of hand-held lactate analyzers against benchtop methods, there was no actual published evidence validating these benchtop methods for use in dogs (Chapter 3). Accordingly, this became the objective of the experimental component of this thesis (Chapter 4).

A partial method validation study was designed to satisfy requirements outlined by the American Society of Veterinary Clinical Pathologists (ASVCP). The objective was to evaluate the analytical performance and equivalency of two commercially available benchtop lactate analyzers: a high-end point-of-care analyzer, the Radiometer ABL 800 (ABL800), and a clinical laboratory analyzer, the Cobas Integra 400 (Cobas400). As a gold-standard, definitive method for lactate measurement is poorly defined, we elected to use canine plasma manually spiked with reference standard sodium lactate as our test medium. After a preliminary assessment of linearity, intra-assay precision, and inter-assay precision using manufacturer controls, we used the spiked plasma to assess linearity, intra-assay precision, accuracy, range and equivalency of the two analyzers.

Both analyzers demonstrated excellent precision. The Cobas400 was accurate over a wider range than the ABL800, however the ABL800 was more accurate within the most clinically relevant range. Method comparison was performed using the Cobas400 as the nominated reference method. Passing-Bablok linear regression and Bland-Altman analysis revealed that although the methods were in agreement, with  $\approx 95\%$  of measured differences falling within  $\pm 1.96$  standard deviations of the mean difference, they cannot be considered equivalent due to the presence of a small but clinically significant amount of constant and proportional bias.

In conclusion, both analyzers demonstrated adequate analytical performance for use in clinical practice and future validation studies. However, they cannot be considered equivalent due to the presence of both constant and proportional bias and should not be used interchangeably. Consequently, analyzer specific reference intervals should be applied, and the same analyzer should be used when serially trending lactate concentrations in an individual patient.

## **DECLARATION PAGE**

I declare that this thesis includes only my original work performed for the completion of the Master of Veterinary Science degree apart from those sections outlined in the preface. Any other material that has been included has been duly acknowledged. Lastly, this thesis is fewer than the maximum word limit of 30 000, exclusive of tables, maps, bibliographies and appendices, as outlined in the University Handbook for the Master of Veterinary Science degree.

Patricia Rosenstein

## **PREFACE**

The content of Chapter 1, including diagrams and tables, has been published in two state of the art reviews in the Journal of Veterinary Emergency and Critical Care (Appendix 1 and 2). These articles were written with the assistance of two co-authors, Dr. Dez Hughes and Dr. Brett Tennent-Brown, who provided roughly 10% of overall content to the articles. The review articles were used as part of my credentials package for the American College of Veterinary Emergency and Critical Care but have not been used towards any other qualifications. All work was completed after enrolment in the MVSc degree.

Third party assistance was provided by Ricardo Maldonado of Power Stats statistical consultancy service (West Ryde, NSW) for statistical analysis in Chapter 4. This study was supported by funding from an internal grant for research masters projects from the University of Melbourne Veterinary Teaching Hospital (AUD\$ 3,500).

## **ACKNOWLEDGEMENTS**

First and foremost, I would like to express sincerest gratitude and appreciation to my primary research supervisor and clinical mentor, Dr. Dez Hughes. Dr. Hughes provided endless support throughout my clinical training, preparation of publications, as well as the development of this thesis. He played a critical role in not only the completion of this thesis but also my success in becoming a diplomate of the American College of Veterinary Emergency and Critical Care.

I would also like to recognize the other members of my research committee. Dr. Natalie Courtman, Dr. Manuel Boller, Dr. Ted Whittem, and Dr. Ken Hinchcliff all provided valuable advice and support throughout the duration of my candidature. Dr. Natalie Courtman in particular went out of her way on several occasions to provide her time, advice and extra resources which were indispensable to this project.

In addition, I would like to acknowledge and express thanks to Dr. Brett Tennent-Brown. Dr. Tennent-Brown was a co-author on two articles that were written and successfully published in the Journal of Veterinary Emergency and Critical Care during my period of enrollment. The articles were ambitious and required a significant amount of dedicated time from Dr. Tennent-Brown in addition to Dr. Hughes, both of whom provided truly invaluable input. Large portions of these articles have been included in my thesis, and the articles can be found in their entirety as cited in Appendix 1 and 2.

Importantly, I absolutely must acknowledge the entire clinical pathology team at the University of Melbourne's Veterinary Teaching Hospital, including Dr. Natalie Courtman, Debra Kirkham, Daniel Pilbeam, Fiona Armour and Amanda Hall. Not only did this wonderful group of people perform all required maintenance and process all samples on the clinical pathology analyzer, but Fiona also volunteered her dogs for blood donation for my project. This endeavour would have been far more challenging if it wasn't for their kindness, support and willingness to donate time for the purpose of my study.

And lastly, my husband, Andrew. Whose kindness, patience and unwavering support during my candidature was simply indescribable.

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#### **LIST OF THIRD PARTY COPYRIGHT MATERIAL**

- Figures 1, 2, 3, 4, 5, 6 and Table 1 were published in: Rosenstein PG, Tennent-Brown BS, Hughes D. State of the Art Review: Clinical use of plasma lactate concentration (Part 1): Physiology, pathophysiology and measurement. J Vet Emerg Crit Care 2018; 28 (2), 85-105.
- Tables 2, 3 and 4 were published in: Rosenstein PG, Tennent-Brown BS, Hughes D. State of the Art Review: Clinical use of plasma lactate concentration (Part 2): Prognostic and diagnostic utility and the clinical management of hyperlactatemia. J Vet Emerg Crit Care 2018; 28 (2), 106-121.

## GENERAL INTRODUCTION

Once considered a simple by-product of anaerobic metabolism, lactate is now recognized to be an important intermediate in cellular bioenergetics. Research into this once misunderstood metabolite has gained momentum over recent decades and lactate is now commonly accepted as a useful tool in both the human and veterinary medical arenas. Clinically, measurement of lactate has demonstrable value within the emergency and critical care discipline. Lactate has been shown to be a valuable triage tool, prognostic indicator and potential therapeutic target.

Several review articles investigating the clinical application of lactate in small animal practice have previously been published.<sup>1-3</sup> These articles, however, provided only a cursory summary of relevant physiology and pathophysiology, and did not incorporate important evidence collected over the past 5-10 years. As such, it was evident that an updated, comprehensive narrative review was needed. This review subsequently led to the recognition of an important void in the veterinary literature. It became apparent that despite widespread incorporation into veterinary emergency and critical care practice, little research had been performed to formally validate benchtop lactate analyzers in dogs. This finding was surprising, given benchtop analyzers have been used as reference analyzers to validate handheld devices. As such, the decision was made to perform a method validation study rather than a clinical trial for this thesis.

This thesis is partitioned into four chapters. Chapter 1 is an expansive, narrative literature review investigating the role of lactate in small animal emergency and critical care medicine which explores the biochemistry, physiology, pathophysiology and clinical application of lactate, focusing on literature specific to dogs, cats and people. Chapter 2 is a targeted review scrutinizing the technical aspects of lactate analysis in dogs. Chapter 3 briefly reviews method validation and the robustness of previously published validation studies investigating the use of lactate analyzers in dogs. Chapter 4 is a validation and comparison study of two benchtop analyzers for the measurement of lactate in canine plasma. In its entirety, this manuscript is intended to provide the veterinary community with a rigorous foundation for both the clinical application of lactate in practice, and for future lactate research. The vast majority of material in chapters 1-3 have been recently published in the Journal of Veterinary Emergency and Critical Care (JVECC) (Appendix 1,<sup>4</sup> Appendix 2<sup>5</sup>).

## CHAPTER 1: CLINICAL USE OF LACTATE IN SMALL ANIMAL PRACTICE

### INTRODUCTION

Experimental studies investigating lactate in dogs began in the 1950s.<sup>6</sup> Clinical investigations, however, did not appear in the literature until the 1990's. Since then, lactate research in companion animals has increased exponentially, paralleling increased practitioner awareness, increased availability of lactate analyzers and research in human medicine. Several review articles exploring the application of lactate in small animal practice have been previously published.<sup>1-3</sup> While these reviews were excellent at the time of publication, they failed to capture important developments in lactate research over the past 5-10 years. In addition, they provided a rather cursory review of pertinent physiology and pathophysiology. This chapter was written with the intention to provide an updated, comprehensive review of the clinical application of lactate in small animal practice, with a particular emphasis on the use of lactate as a prognostic indicator in dogs.

### HISTORY

Lactic acid was first documented in sour milk by Karl Wilhelm Scheele in 1780.<sup>8,9</sup> In 1843, Johann Joseph Scherer identified lactic acid in human blood after death,<sup>8</sup> and in 1878 George Salomon documented lactate in the blood of people suffering from a range of illnesses including anemia, congestive heart failure, pericarditis and neoplasia.<sup>8</sup> In the mid 1900's Huckabee performed a number of landmark studies characterizing the relationship between lactate and pyruvate under various conditions of reduced oxygen availability.<sup>6,10-15</sup> In 1964, Broder and Weil showed that, in undifferentiated shock, people with blood lactate concentrations greater than 4 mmol/L had a markedly lower survival than those with blood lactate concentrations less than 4 mmol/L.<sup>16</sup> These early investigations formed the essential foundation for our current understanding of lactate and hyperlactatemia.

### PHYSIOLOGY

#### Biochemistry

The terms lactate and lactic acid are often used erroneously as synonyms. Lactate, ( $\text{C}_3\text{CH}(\text{OH})\text{COO}^-$ ), is an anion and conjugate base to lactic acid, ( $\text{C}_3\text{CH}(\text{OH})\text{COOH}$ ).<sup>17</sup> (Figure 1) As the pKa of lactic acid is 3.8, at physiologic pH, lactic acid is essentially fully dissociated into lactate anions and protons ( $\text{H}^+$ ).<sup>18</sup> Lactic acid is not, however, produced *in vivo*.<sup>19-21</sup> Instead, lactate is produced directly in its ionized form during carbohydrate metabolism, and the production of lactate from pyruvate involves the consumption of protons rather than their release.<sup>19</sup>

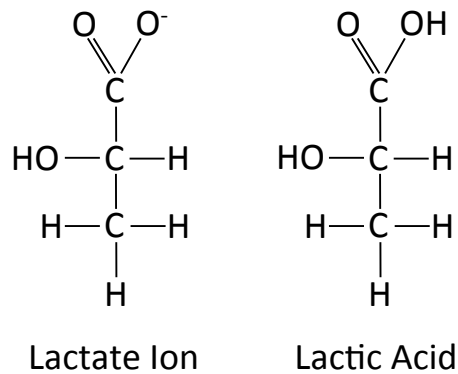


Figure 1: Lactate ion and lactic acid (Rosenstein 2018)

Lactate exists in two stereoisomeric forms, L-lactate and D-lactate. (Figure 2) In health, L-lactate accounts for > 99% of total body lactate and is the isomer of major physiological significance.<sup>18,22-24</sup> D-lactate is formed either by the glyoxalase pathway or produced by commensal bacteria in the mammalian gastrointestinal tract and absorbed into circulation.<sup>22</sup> Unless otherwise specified, in this manuscript, “lactate” will be used in reference to L-lactate.

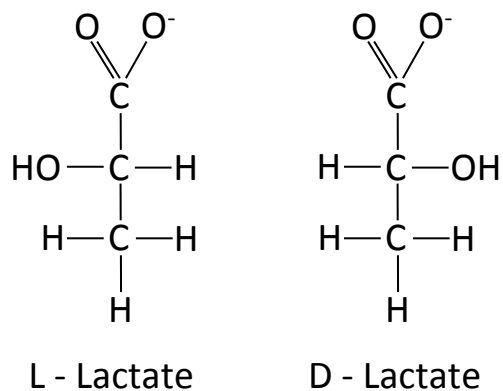


Figure 2: L-lactate and D-lactate (Rosenstein 2018)

The production of lactate is dependent on perhaps the most proverbial of metabolic pathways, glycolysis. Glycolysis is the cytosolic, biochemical cascade by which one 6-carbon glucose molecule is broken down into two 3-carbon molecules of pyruvate, two molecules of ATP and two molecules of reduced nicotinamide adenine dinucleotide (NADH). This process involves 11 distinct enzymatic reactions (Figure 3).

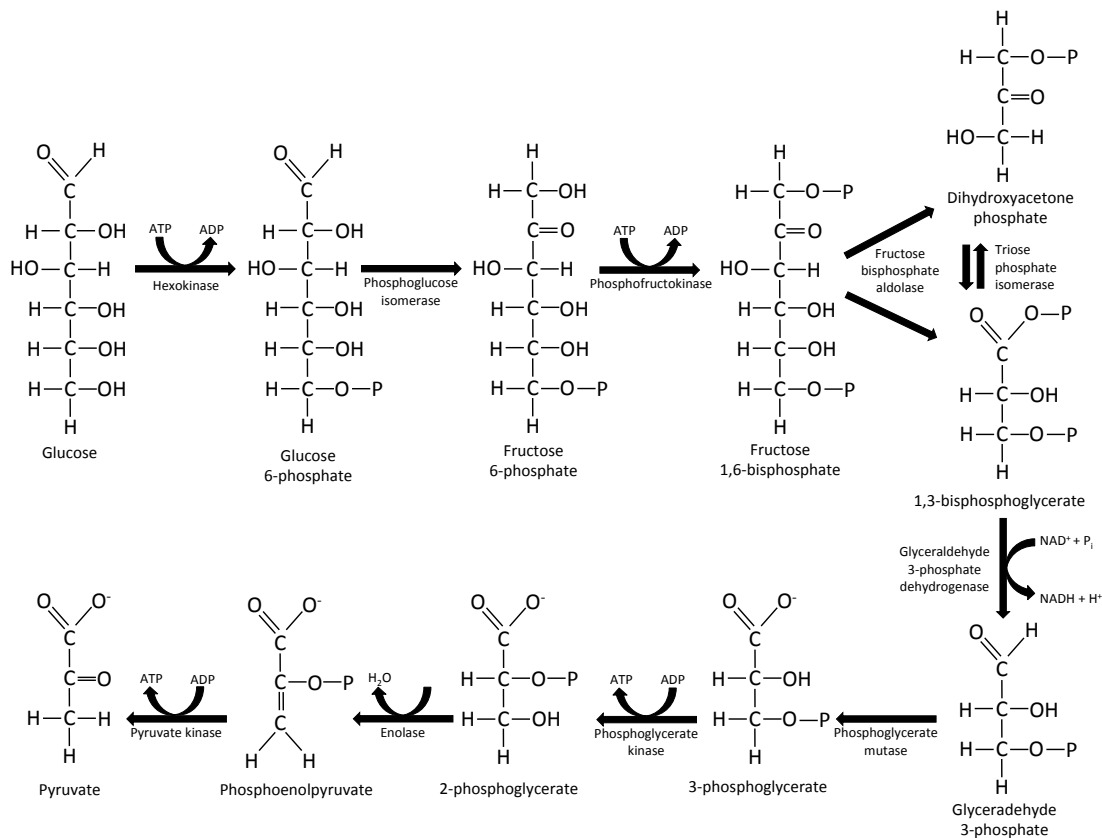


Figure 3: The 11 steps of glycolysis (Rosenstein 2018)

Glycolysis requires a constant supply of glucose and oxidized nicotinamide adenine dinucleotide (NAD<sup>+</sup>) to produce ATP but does not require oxygen. The ultimate product of glycolysis, pyruvate, is transported into the mitochondrion<sup>25</sup> where it undergoes decarboxylation to produce Acetyl-CoA.<sup>21</sup> This reaction is irreversible, requires NAD<sup>+</sup>, and is catalyzed by the pyruvate dehydrogenase complex. Acetyl-CoA then proceeds through the tricarboxylic acid (TCA) cycle to produce CO<sub>2</sub>, NADH and FADH<sub>2</sub>.<sup>26</sup> Protons from NADH and FADH<sub>2</sub> create the proton gradient required for the production of adenosine triphosphate (ATP) by the electron transport chain (ETC). When glycolysis, the TCA cycle and the ETC are combined, the oxidation of one molecule of glucose produces approximately 36 molecules of ATP.<sup>27,28</sup>

Under healthy, resting conditions, approximately 10% of pyruvate is converted into lactate by lactate dehydrogenase (LDH).<sup>10,21,29-31</sup> This is a reversible, cytosolic reaction during which NADH is oxidized to NAD<sup>+</sup> (Figure 4).

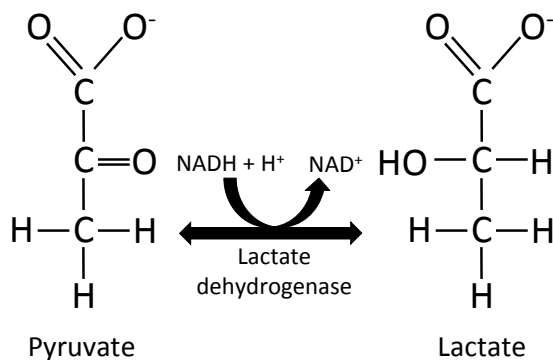


Figure 4: Lactate to pyruvate catalyzed by lactate dehydrogenase (Rosenstein 2018)

When oxygen demand exceeds supply,  $\text{NAD}^+$  stores become depleted, pyruvate and  $\text{NADH}$  accumulate in the cytosol and LDH activity is upregulated.<sup>28,32</sup> Lactate formation reduces the cytosolic concentrations of pyruvate and  $\text{H}^+$  while replenishing  $\text{NAD}^+$  enabling glycolysis to continue supplying ATP.<sup>32-34</sup> Once oxygen supply is restored, LDH transforms lactate back into pyruvate, which can enter the TCA cycle or be used for gluconeogenesis. Fundamentally, lactate is a highly conserved, easily transported energy storage molecule; a form of energy currency that is transferred as required to suit metabolic demands. The metabolic acidosis that occurs with hyperlactatemia is the result of ATP hydrolysis without concurrent proton consumption by the ETC and subsequent proton accumulation.<sup>19,35-37</sup>

Despite this, as a strong anion, lactate does have an acidifying effect in a manner similar to chloride as per Stewart's physicochemical approach.<sup>17,29,38-40</sup> This is because an increase in lactate causes a decrease in the strong ion difference (SID)<sup>41,42</sup> (Figure 5). In accordance with the law of electroneutrality, decreasing the SID results in a proportionate increase in  $[\text{H}^+]$  and therefore, acidosis.<sup>43-45</sup>

$$[\text{SID}^+] = [\text{Na}^+] + [\text{K}^+] + [\text{Ca}^{2+}] + [\text{Mg}^{2+}] - [\text{Cl}^-] - [\text{lactate}]$$

Figure 5: Strong ion difference<sup>41,42</sup>

The quantitative approach to acid base suggests that for every 1 mmol/L increase in lactate, the standard base excess will decrease by 1 unit.<sup>46</sup> In light of these different approaches, it is not surprising that the precise relationship between lactate and acidosis is complex and controversial.<sup>20,34,36,44,47-50</sup> The correlation between acidosis and lactate concentration is particularly inconsistent in the presence of aerobic hyperlactatemia.<sup>45,46,51-54</sup>

## Lactate Transport

Lactate transport across cell membranes occurs predominantly via facilitated passive transport by proton-linked monocarboxylate transporters (MCT) and sodium-coupled MCTs.<sup>55,56</sup> At least 14 MCTs have been identified although MCT1 through MCT4 are considered the most important in mammalian tissues.<sup>56-58</sup> These MCTs are variably expressed in different tissues and are relatively non-specific, moving a number of different substrates including lactate, pyruvate, acetate, propionate, butyrate, acetoacetate and  $\beta$ -hydroxybutyrate down concentration gradients.<sup>55,57</sup> Erythrocyte MCT expression and activity vary between species.<sup>56</sup> This explains the species-dependent variation seen between whole blood and plasma or serum lactate concentrations. The MCTs are variably upregulated in response to stimuli including exercise, hypoxia, and bacterial infection.<sup>55,59</sup> Simple diffusion and anionic exchange also occur and can significantly contribute to lactate transport in certain species such as ruminants.<sup>39,60,61</sup>

Lactate transporters also play an essential role in “lactate shuttles,” a form of energy currency exchange between different cells and tissue types.<sup>62-64</sup> Lactate shuttles have been shown to exist in the brain, striated muscle, liver, kidneys and myocardium.<sup>28</sup> The Cori cycle is an example of a lactate shuttle whereby lactate is produced in a distant tissue and then transferred to the liver or kidney for gluconeogenesis.<sup>28,64</sup>

## Lactate Production, Metabolism, and Excretion

While almost all tissues are capable of producing and consuming lactate, the majority of lactate produced at rest comes from skeletal muscle (40-50%),<sup>29,32,55</sup> the brain (13%),<sup>32</sup> erythrocytes (23%),<sup>65,66</sup> and adipose tissue (variable)<sup>32,55</sup> by virtue of their inherent production capacities and mass. Other lactate producing tissues include the renal medulla, gastrointestinal tract, skin, white blood cells, and platelets.<sup>55,65,67</sup>

Erythrocytes, leukocytes (predominantly neutrophils) and platelets are responsible for 80, 13 and 7% of lactate production in blood, respectively.<sup>68,69</sup> Glycolysis and lactate production are increased during the oxidative burst of neutrophils<sup>69</sup> and experimentally induced sepsis has been shown to increase leukocyte production of lactate 6-fold.<sup>68</sup>

The most important lactate consuming tissues are the liver (18-50%),<sup>29,32,70</sup> the renal cortex (20-30%)<sup>32,67,70</sup> and the myocardium (5-15%).<sup>55</sup> The liver has a particularly large reserve capacity for lactate metabolism.<sup>21,71,72</sup> Hepatic lactate uptake is, however, a

saturable process.<sup>21,73</sup> During hyperlactatemia, certain tissues, including the brain and skeletal muscle, substantially increase lactate consumption, sometimes even preferentially to readily available glucose.<sup>55,27,74</sup> As a valuable energy substrate, lactate is filtered by the glomerulus but avidly reabsorbed by the renal proximal convoluted tubule.<sup>21,67,75</sup>

## **PATHOPHYSIOLOGY**

Lactic acidosis has been divided into two broad categories: Type A, due to an insufficient oxygen supply, and Type B, in the face of apparently adequate oxygen availability<sup>76</sup> (Table 1 and Figure 6). Patients can experience Type A and Type B hyperlactatemia concurrently. Type A hyperlactatemia can be relative, resulting from increased oxygen demand, or absolute, resulting from inadequate oxygen delivery. Type B hyperlactatemia has conventionally been subdivided into three categories: B1, associated with underlying disease, B2, associated with drugs or toxins, and B3, resulting from congenital errors in metabolism.

| Type A                                        |                               | Type B                                    |                                              |                                |                                     |
|-----------------------------------------------|-------------------------------|-------------------------------------------|----------------------------------------------|--------------------------------|-------------------------------------|
| Relative                                      | Absolute                      | B1: Disease                               | B2: Drugs/Toxins                             |                                | B3: Congenital                      |
| Exercise <sup>§,+</sup>                       | Hypo-perfusion <sup>§,+</sup> | Malignancy <sup>∞,⌘,*</sup>               | Glucocorticoids <sup>∞,⌘,+</sup>             | Epinephrine <sup>∞,⌘,+</sup>   | MELAS <sup>*</sup>                  |
| Muscle tremors <sup>§</sup>                   | Severe anemia <sup>§</sup>    | Diabetes Mellitus <sup>∞,⌘,*</sup>        | Acetylcholinesterase inhibitors <sup>§</sup> | Acetaminophen <sup>∞,⌘,*</sup> | Mitochondrial myopathy <sup>*</sup> |
| Shivering <sup>§</sup>                        | Severe hypoxemia <sup>§</sup> | Hepatic failure <sup>∞,⌘</sup>            | Fructose <sup>∞</sup>                        | Salicylates <sup>∞,⌘,¶,*</sup> | PDH deficiency <sup>⌘</sup>         |
| Seizures <sup>§</sup>                         | Carbon monoxide <sup>§</sup>  | Thiamine deficiency <sup>⌘,¶</sup>        | Xylitol <sup>∞</sup>                         | β-agonists <sup>∞,⌘,+</sup>    |                                     |
| Struggling <sup>§,+</sup>                     |                               | Hyperthyroidism <sup>∞,⌘</sup>            | Sorbitol <sup>∞</sup>                        | Cocaine <sup>§,∞,+</sup>       |                                     |
|                                               |                               | Microcirculatory dysfunction <sup>§</sup> | Glucose <sup>∞</sup>                         | Methylxanthines <sup>∞,+</sup> |                                     |
|                                               |                               | Cytopathic hypoxia <sup>∞,⌘,*</sup>       | Strychnine <sup>§</sup>                      | Cyanide <sup>*</sup>           |                                     |
|                                               |                               | Impaired gluconeogenesis <sup>❖</sup>     | Biguanides <sup>*</sup>                      | Nitroprusside <sup>*</sup>     |                                     |
|                                               |                               | Alkalosis <sup>∞</sup>                    | Linezolid <sup>*</sup>                       | Ethylene glycol <sup>⌘,#</sup> |                                     |
|                                               |                               | SIRS/Sepsis <sup>§,⌘,+,*</sup>            | Isoniazid <sup>§</sup>                       | Methanol <sup>§,*,#</sup>      |                                     |
|                                               |                               | Pheochromocytoma <sup>∞,+</sup>           | NRTI's <sup>*</sup>                          | Propylene glycol <sup>❖</sup>  |                                     |
|                                               |                               |                                           | Lactulose <sup>❖</sup>                       | Ethanol <sup>#</sup>           |                                     |
|                                               |                               | 5-Fluorouracil <sup>¶</sup>               | Propofol <sup>*</sup>                        |                                |                                     |
| Miscellaneous: D-lactic acidosis <sup>❖</sup> |                               |                                           |                                              |                                |                                     |

Table 1 – Type A vs. B classification system for hyperlactatemia with proposed mechanisms of hyperlactatemia corresponding to Figure 6<sup>4</sup>

§ - Inadequate oxygen supply, \* - Dysfunction of the electron transport chain, ∞ - Upregulation of glycolysis, ⌘ - Inhibition of the pyruvate dehydrogenase complex, + - Upregulation of the Na<sup>+</sup>-K<sup>+</sup>-ATPase and associated glycolytic chain, # - Increased NADH/NAD<sup>+</sup> ratio, ¶ - Inhibition of the tricarboxylic acid cycle, ❖ - Direct production or lack of consumption of lactate. MELAS - Mitochondrial encephalomyopathy with lactic acidosis and stroke syndrome, SIRS – Systemic inflammatory response syndrome, NRTI - Nucleoside HIV reverse transcriptase inhibitors.

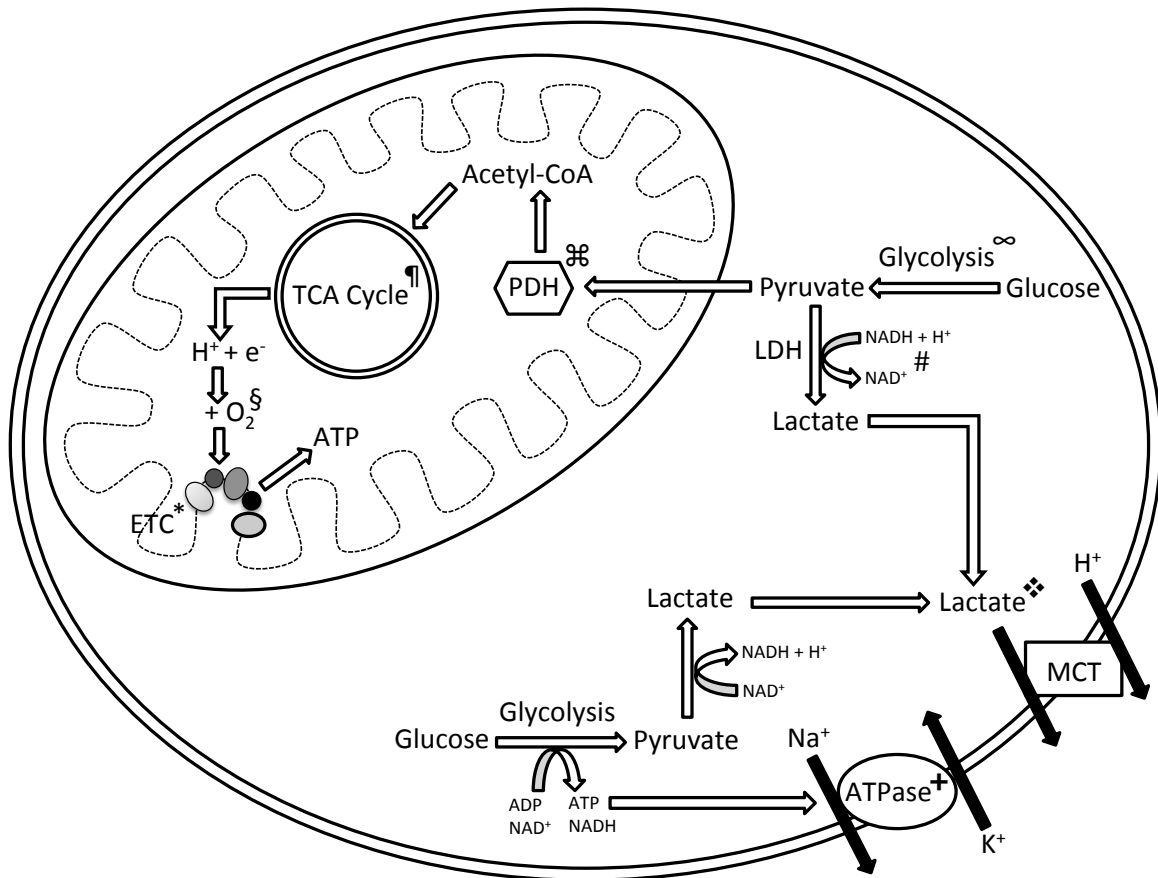


Figure 6: Sites of action of the various causes of hyperlactatemia corresponding to Table 1.  
H<sup>+</sup> - proton, e<sup>-</sup> - electron<sup>4</sup>

### Relative Type A Hyperlactatemia

Hyperlactatemia from increased oxygen demand can occur due to exercise, seizure activity, shivering, trembling or struggling. A 5-minute spray bath in cats dramatically increased blood lactate concentrations (mean  $\approx 7.0$  mmol/L),<sup>77</sup> while mild to moderate struggling associated with restraint for venipuncture did not have an appreciable effect.<sup>78</sup> Exercise induced hyperlactatemia can be marked but is highly variable. Maximal lactate concentrations after exercise range from 6.3 mmol/L in Labrador Retrievers during field training,<sup>79</sup> to 14.6 mmol/L in dogs after agility testing,<sup>80,81</sup> to over 30 mmol/L in racing greyhounds.<sup>82-87</sup> In one study, healthy Labrador Retrievers were exercised for up to 30 minutes on a treadmill to perceived exhaustion without an appreciable increase in blood lactate,<sup>88</sup> while in another, healthy Labrador Retrievers were exercised for 10 minutes and demonstrated post exercise lactate concentrations of  $3.57 \pm 2.22$  mmol/L (mean  $\pm$  SD) with a maximum concentration of 9.86 mmol/L observed in a single animal.<sup>89</sup>

In healthy animals, lactate concentrations fall rapidly following cessation of muscle activity with an estimated half-life of 20-60 minutes.<sup>83,87,89-93</sup> Seizure-induced hyperlactatemia results primarily from vigorous muscle activity and is associated with a similar half-life.<sup>29,91,94</sup> A persistent increase in lactate concentration following cessation of seizure activity is cause for concern.

### **Absolute Type A Hyperlactatemia**

Hyperlactatemia from decreased oxygen delivery is observed with shock, local hypoperfusion, severe anemia, severe hypoxemia, and carbon monoxide poisoning.

#### *Shock*

Shock is likely to be the most common cause of pathologic hyperlactatemia in veterinary emergency and critical care practice. By definition, shock is associated with inadequate oxygen delivery to the tissues leading to impaired mitochondrial respiration and increased anaerobic metabolism. The onset of hyperlactatemia relative to oxygen delivery is similar in hypovolemic, cardiogenic and obstructive shock,<sup>95,96</sup> but occurs earlier in maldistributive shock due to impaired oxygen extraction from mitochondrial and microcirculatory dysfunction.<sup>95-98</sup> Shock has traditionally been identified by physical exam parameters including tachycardia, hypotension, depressed mentation and inadequate urine output. Tissue hypoperfusion can, however, be present despite normal standard hemodynamic variables, a phenomenon termed “occult shock”.<sup>99</sup> In a recent clinical trial, dogs presenting in hypovolemic or septic shock were resuscitated to satisfy traditional hemodynamic targets. In this study, 6/30 dogs had persistently elevated lactate concentrations and 11/30 dogs had persistently low ScvO<sub>2</sub> (< 70%) suggesting ongoing tissue hypoperfusion despite normalization of routine hemodynamic parameters.<sup>100</sup> The hyperlactatemia of shock is unlikely to be solely due to impaired oxygen delivery leading to increased anaerobic metabolism. In several experimental animal models and human clinical trials, hyperlactatemia in shock was found to be independent of oxygen consumption and delivery.<sup>98,101-106</sup> Several causes of Type B hyperlactatemia, to be elaborated on in later sections, are believed to be important contributors to shock-associated hyperlactatemia.

#### *Regional Ischemia*

Hyperlactatemia is considered a non-specific indicator of mesenteric ischemia as peripheral blood lactate concentrations can also increase with other abdominal crises (i.e. bacterial peritonitis, acute pancreatitis) and may reflect global hypoperfusion more than regional ischemia.<sup>107-109</sup> The ability of plasma lactate concentration to differentiate patients with gastrointestinal ischemia from those without varies between studies. For example, in one study investigating dogs with gastric dilatation and volvulus, 74% (23/31) of dogs with lactate greater than 6.0 mmol/L had gastric necrosis,<sup>110</sup> whereas in another, only 33% (8/24) of dogs with lactate greater than or equal to 6 mmol/L had gastric necrosis.<sup>111</sup> Furthermore, peripheral lactate concentrations can remain normal despite significant

splanchnic ischemia.<sup>112</sup> As peripheral blood lactate concentrations are inconsistently and non-specifically elevated in the presence of abdominal disease, caution must be exercised when using blood lactate concentrations to rule in or rule out intra-abdominal catastrophes.<sup>113</sup>

### *Anemia and Hypoxemia*

The correlation between anemia and hyperlactatemia is highly dependent on chronicity of disease. Animals with chronic anemia can remain eulactatemic even when anemia is severe.<sup>114</sup> In contrast, animals with anemia resulting from acute hemorrhage or acute, severe hemolysis can develop clinically significant hyperlactatemia.(Holahan 2010) In experimental animal models of acute dilutional anemia, hyperlactatemia did not develop until packed cell volume was below 15%.<sup>115-117</sup> Hyperlactatemia from hypoxemia alone is likely rare in veterinary medicine, as  $P_{aO_2}$  values need to be 25-40 mmHg before lactate concentrations begin to rise.<sup>116,118</sup>

### *Carbon Monoxide*

Carbon monoxide binds to hemoglobin with a greater affinity than oxygen to produce carboxyhemoglobin which inhibits effective oxygen transport.<sup>119</sup> Carbon monoxide also shifts the oxyhemoglobin curve to the left, further reducing oxygen delivery, and directly induces cellular damage and oxidative stress.<sup>119</sup> Hyperlactatemia from carbon monoxide toxicity is suspected to result primarily from tissue hypoxia, although mitochondrial dysfunction and catecholamine effects, which will be discussed further in later sections, may also contribute.<sup>55</sup> In keeping with a multifactorial pathogenesis, lactate concentration is variably correlated with carboxyhemoglobin concentrations as well as the duration of carbon monoxide exposure.<sup>120-122</sup>

## **Type B1 Hyperlactatemia – Associated with Underlying Disease**

### *Malignancy*

The phenomenon of altered cellular metabolism in cancer cells was first characterized by Warburg in 1929, and has subsequently been referred to as the “Warburg effect.”<sup>55,123</sup> A variety of potential mechanisms for malignancy-associated hyperlactatemia have been identified including: increased production of glycolytic enzymes by hypoxia-inducible factors 1 and 2,<sup>123-125</sup> increased production of glycolysis intermediates via upregulation of the pentose phosphate pathway,<sup>55,126</sup> mitochondrial dysfunction, reduced hepatic clearance, and malnutrition causing thiamine deficiency.<sup>17,29</sup> Malignant cells preferentially use glycolytic pathways despite readily available oxygen<sup>123</sup> and lactate may alter the tumor micro-environment in a manner that inhibits destruction of neoplastic cells.<sup>123,127-129</sup> The inhibition of enzymes involved in lactate transport and metabolism has been found to inhibit tumor growth in various neoplastic cell lines. Regulation of lactate metabolism is, consequently, now a target of modern cancer therapy.<sup>55,123,127-130</sup>

In people, hyperlactatemia has been associated with neoplasia of hematologic origin, lung cancer, prostate cancer, breast cancer, endometrial carcinoma, melanoma, prostate cancer, and cholangiocarcinoma.<sup>55,30,131</sup> In addition, pheochromocytoma is associated with catecholamine-induced hyperlactatemia.<sup>132,133</sup> In a retrospective study including 50 dogs with lymphoma, hyperlactatemia was documented in 20 cases, although 15 had concurrent confounding pathologies such as sepsis or hepatic dysfunction.<sup>134</sup> An association between hyperlactatemia and canine neoplasia may exist but is likely to be uncommon.<sup>134,135</sup> In a clinical setting, neoplasia-associated hyperlactatemia should be considered a diagnosis of exclusion.

### *Diabetes Mellitus*

Altered carbohydrate metabolism in patients with diabetes mellitus occurs due to upregulated glycolysis, impaired glycogenesis, reduced PDH activity, and reduced oxidative metabolism.<sup>55,136,137</sup> Lactate concentrations in people with Type 1 and Type 2 diabetes mellitus are approximately 0.3-1.0 mmol/L higher than in non-diabetics.<sup>136,137</sup> Hyperlactatemia is commonly observed in diabetic ketoacidosis due to a combination of hypovolemia and altered carbohydrate metabolism: median 3.5 mmol/L (range: 1.2-8.3 mmol/L).<sup>138</sup> People with diabetic ketoacidosis also have significantly higher D-lactate concentrations (mean  $\pm$  SD: 2.14  $\pm$  2.18 mmol/L) compared to diabetic, non-ketotic patients (mean  $\pm$  SD: 1.32  $\pm$  0.77 mmol/L) and controls (mean  $\pm$  SD: 1.03  $\pm$  2.18 mmol/L).<sup>22</sup> This is speculated to be the result of increased methylglyoxal production and breakdown due to altered glucose and increased ketone metabolism.<sup>22</sup> Dogs with diabetes mellitus also have statistically higher lactate than normal dogs: median 2.0 mmol/L (range 0.9-5.8 mmol/L) vs. median 1.0 mmol/L (range 0.6-1.3 mmol/L) respectively.<sup>139</sup> The hyperlactatemia identified in these diabetic dogs was speculated to be the result of dehydration, decreased tissue perfusion, and impaired lactate metabolism.<sup>139</sup> In agreement with this theory, a recent prospective study including mostly normovolemic dogs did not find a significant difference in plasma lactate between 25 newly diagnosed diabetics (median: 1.69, P<sub>25-75</sub>: 1.09-2.40), 25 diabetics being treated with insulin (median: 2.27, P<sub>25-75</sub>: 1.44-2.90), 25 with diabetic ketoacidosis (median: 2.40, P<sub>25-75</sub>: 1.58-3.01) and 25 non-diabetics (median: 2.23 mmol/L, P<sub>25-75</sub>: 1.46-2.83 mmol/L).<sup>140</sup> Although cats with diabetic ketoacidosis have been shown to have significantly higher D-lactate concentrations than controls,<sup>141</sup> a significant increase in L-lactate has not been identified in cats with diabetes mellitus or diabetic ketoacidosis.<sup>141,142</sup>

### *Hepatic and Renal Disease*

In people, severe liver disease is rarely associated with hyperlactatemia unless they are acutely challenged by a lactate load or are experiencing concurrent hypoperfusion.<sup>29,54,143,144</sup> People with chronic liver disease demonstrate slowed lactate metabolism, but peak lactate values are often similar to healthy controls.<sup>54,145,146</sup> In the presence of severe hepatic dysfunction or failure the liver can become a net lactate

producer causing hyperlactatemia.<sup>54,147</sup> Postulated mechanisms for hepatic lactate production include glycolytic upregulation, reduced PDH activity, and impaired hepatic gluconeogenesis.<sup>54,55</sup> Although lactic acidosis is considered a rare complication of hematologic neoplasia, in one literature review, hepatic involvement was identified in 80% (42/52) of hyperlactatemic people with leukemia or lymphoma.<sup>148</sup> Similarly, 100% of dogs (10/10) with lymphoma and documented hepatic involvement were hyperlactatemic although 5/10 suffered from concurrent hemorrhage, sepsis or volume depletion.<sup>134</sup> In a canine model of experimentally induced septic shock, septic dogs demonstrated both increased hepatic lactate production and reduced hepatic lactate extraction compared to non-septic controls following lactic acid administration.<sup>149</sup> Although the kidneys play an important role in lactate metabolism, reports of hyperlactatemia from acute or chronic renal failure are scarce without contributing factors such as the use of biguanide anti-hyperglycemics or hypovolemia.<sup>67,150-152</sup>

### *Thiamine Deficiency*

Thiamine deficiency (vitamin B<sub>1</sub>) is an uncommon but well documented cause of hyperlactatemia.<sup>153-160</sup> Thiamine plays an important role in several metabolic pathways after conversion to thiamine pyrophosphate.<sup>55</sup> Thiamine pyrophosphate is a cofactor in the PDH complex,  $\alpha$ -ketoglutarate dehydrogenase in the TCA cycle, transketolase in the pentose phosphate pathway, and ketoacid dehydrogenase.<sup>153,154,158,161,162</sup> Thiamine deficiency, also known as beriberi disease, leads to compromised cellular bioenergetics and the accumulation of pyruvate and lactate.<sup>153,156</sup> Thiamine deficiency can be caused by total parenteral nutrition without thiamine supplementation, malignancy, sepsis, critical illness, chronic malnutrition, chronic pyloric outflow obstruction, and hyperthyroidism.<sup>17,156,159,163,164</sup> Thiamine deficiency manifests clinically in people as hyperlactatemia, vasodilation, peripheral edema, cardiac dysfunction, vomiting, peripheral neuropathy and Wernicke's encephalopathy.<sup>55,153,156,157</sup> In dogs and cats, thiamine deficiency has been reported in association with the ingestion of fish high in thiaminase,<sup>165,166</sup> food with inactivated thiamine from processing,<sup>167,168</sup> and sulfite-preserved meat.<sup>153,169,170,171</sup> Thiamine deficiency in dogs and cats typically manifests as cervical ventroflexion, ataxia, mydriasis, depressed mentation and seizure activity.<sup>153,165-171</sup>

### *Hyperthyroidism*

Hyperthyroidism causes an increased basal metabolic rate leading to increased carbohydrate metabolism and hyperlactatemia through upregulation of glycolysis and the hexose monophosphate pathway.<sup>142,172-176</sup> In addition, hyperthyroidism and thyroid storm are considered risk factors for thiamine deficiency due to depletion of thiamine stores.<sup>164,173,177</sup> One study reported higher serum lactate concentrations in cats with hyperthyroidism than in healthy cats or cats with diabetes (mean $\pm$ SD of 5.0 $\pm$ 3.0 mmol/L and 3.8 $\pm$ 2.2 mmol/L respectively).<sup>142</sup>

### *Microcirculatory Dysfunction*

Microcirculatory dysfunction is considered the most important cause of impaired oxygen extraction in maldistributive shock and is likely an important cause of hyperlactatemia in this patient group.<sup>96,178,179</sup> Sepsis enhances nitric oxide production, alters the neurohormonal control of endothelial and smooth muscle cells, reduces erythrocyte flexibility, and activates leukocytes resulting in marked microcirculatory heterogeneity.<sup>178-182</sup> Erythrocyte aggregation, leukocyte adhesion, local edema and microvascular thrombosis also impair microcirculatory flow.<sup>96,179</sup> Microcirculatory shunting likely contributes to the concurrent hyperlactatemia and elevated SvO<sub>2</sub> seen in sepsis.<sup>181</sup> One of the most important features of microcirculatory dysfunction is that it can occur independently of macrocirculatory hemodynamic variables.<sup>182-184</sup> This means that normalization of vital parameters does not necessarily imply normalization of perfusion at the tissue level. Microcirculatory dysfunction has been correlated with hyperlactatemia in people with sepsis despite normal hemodynamic parameters, supporting the concept that circulatory shunting at the capillary level may lead to local, heterogeneous tissue hypoxia and hyperlactatemia despite apparently adequate oxygen delivery.<sup>185</sup>

### *Altered Cellular Respiration*

Impaired cellular respiration, also known as cytopathic hypoxia, can lead to hyperlactatemia by a number of different pathways including mitochondrial dysfunction, upregulation of glycolysis, and impaired gluconeogenesis.<sup>186</sup> Mitochondrial dysfunction has been implicated as a cause of aerobic hyperlactatemia particularly in the presence of severe inflammation, sepsis or oxidative stress.<sup>187</sup> Mitochondrial respiration can fail as a result of direct cytochrome inhibition, impaired mitochondrial regeneration, or due to a loss of the normal electrochemical gradient between the mitochondrial matrix and intermembrane space resulting in impaired ATP production.<sup>26,188-191</sup> In addition, mitochondrial respiration can be compromised by dysfunction of the pyruvate dehydrogenase complex (PDH).<sup>29,55,161,188,189,192</sup> Alkalosis stimulates the rate limiting glycolytic enzyme phosphofructokinase, subsequently increasing pyruvate and therefore lactate concentrations.<sup>1,29,54,193</sup> Similarly, the administration of glucose, increased NADH concentrations and ATP depletion promote lactate formation via concentration dependent effects.<sup>29,55,194,195</sup>

### *Catecholamines*

Adrenergic stimulation by catecholamines, particularly epinephrine, is likely to be an important cause of clinical hyperlactatemia.<sup>63,196</sup> Epinephrine administration is associated with a progressive, dose-related increase in lactate due to increased cyclic adenosine monophosphate production (cAMP) via  $\beta_2$ -adrenergic stimulation.<sup>197</sup> This leads to enhanced glycogenolysis, glycolysis, lipolysis, and stimulation of Na<sup>+</sup>-K<sup>+</sup>-ATPase pumps.<sup>198-204</sup> Na<sup>+</sup>-K<sup>+</sup>-ATPase pumps are associated with lactate-producing glycolytic pathways that are not coupled to mitochondrial respiration (Figure 6).<sup>201,205-212</sup>

There is compelling evidence to support the upregulation of skeletal muscle Na<sup>+</sup>-K<sup>+</sup>-ATPase associated glycolysis as a clinically significant source of lactate in septic and hemorrhagic shock, as well as exercise-related hyperlactatemia.<sup>196,200,201,206,208,218-224</sup> Not only does plasma lactate correlate with catecholamine concentrations,<sup>86,89,217,225-227</sup> but both selective Na<sup>+</sup>-K<sup>+</sup>-ATPase and adrenergic blockade have been shown to prevent or decrease lactate concentrations in hemorrhagic shock,<sup>200,216,228-232</sup> septic shock<sup>103,196,201</sup> and during exercise.<sup>222,233,234</sup> Hyperlactatemia has even been documented in healthy university students following a university exam.<sup>235</sup> Catecholamine-induced hyperlactatemia likely explains the hyperlactatemia seen with pheochromocytoma<sup>236-238</sup> and acute asthmatic crisis (from endogenous catecholamine release as well as  $\beta_2$  adrenergic agonist therapy).<sup>239-242</sup>

## **Type B2 Hyperlactatemia – Associated with Drugs or Toxins**

### *Acetaminophen and Salicylates*

Acetaminophen toxicity causes hyperlactatemia by impairing mitochondrial respiration, by causing hepatic dysfunction leading to impaired lactate clearance, and by causing methemoglobinemia.<sup>243,244</sup>

Salicylates decrease the availability of CoA, inhibit succinate dehydrogenase and alpha-ketoglutarate dehydrogenase.<sup>29,30,245</sup> They also increase the permeability of the inner mitochondrial membrane leading to uncoupling of oxidative phosphorylation and stimulate phosphofructokinase all of which lead to increased lactate production.<sup>29,30,245</sup> In people, hyperlactatemia associated with salicylate toxicity is usually mild.<sup>246</sup>

### *Antimicrobials (Linezolid, Isoniazid)*

Linezolid, an oxazolidinone antibiotic, has been associated with hyperlactatemia via impaired biogenesis of mitochondrial proteins resulting in impairment of complexes I, III and IV of the electron transport chain.<sup>55,245,247-249</sup> In veterinary medicine, routine use of linezolid is discouraged as it is an important antimicrobial for the treatment of multidrug resistant bacteria in people.

Isoniazid is an anti-mycobacterial used for the treatment and prevention of tuberculosis. Isoniazid has a narrow margin of safety, and toxicity is associated with severe, life threatening consequences including refractory seizure activity and coma.<sup>245,250</sup> The associated hyperlactatemia can be severe, resulting from increased muscle activity associated with protracted seizure activity.<sup>245,250,251</sup>

### *Biguanides anti-hyperglycemic agents (Phenformin, Metformin)*

Phenformin and metformin are oral anti-hyperglycemic agents traditionally used in people with type II diabetes mellitus. Phenformin's molecular properties enable it to bind to mitochondrial membranes where it inhibits the electron transport chain resulting in hyperlactatemia.<sup>21,150</sup> Phenformin was taken off the market in many countries in the 1970's due to unacceptably high rates of life threatening phenformin-induced lactic acidosis.<sup>245,252</sup>

Metformin is less lipophilic and does not appear to be able to bind to mitochondrial membranes as well as phenformin.<sup>55,150,252-254</sup> While metformin-associated lactic acidosis, also known as MALA, remains the most prevalent serious adverse reaction associated with metformin use (18%)<sup>255</sup> MALA is uncommon and of similar incidence to lactic acidosis in diabetic patients that are not receiving metformin.<sup>252,254,255</sup> Furthermore, in over 90% of cases of MALA, patients suffered from concurrent additional risk factors for lactic acidosis such as heart failure, renal failure, hepatic dysfunction, or shock.<sup>150,245,254-258</sup> MALA is, however, recognized in people following metformin overdose.<sup>259,260</sup> While most cases of metformin toxicosis reported to the ASPCA Animal Poison Control Center involve dogs that have ingested their owners' medication, MALA has not yet been documented.<sup>261</sup> The toxic dose of metformin in dogs and cats is not known, but current recommendations are to monitor lactate levels when nearing 200 mg/kg.<sup>261</sup>

### *Adrenergic Agonists and Sympathomimetics*

Hyperlactatemia also occurs with drugs and toxins that cause adrenergic stimulation including:  $\beta_2$ -receptor agonists (terbutaline, albuterol, salbutamol), methylxanthines (theophylline, theobromine), and cocaine.<sup>242,262-265</sup> Terbutaline, albuterol and other  $\beta_2$ -agonists cause hyperlactatemia as previously described.<sup>242,263,266</sup> The methylxanthines promote hyperlactatemia by increasing plasma catecholamine levels, inhibiting cAMP breakdown enhancing  $\beta$ -receptor effects, and by directly stimulating the Na<sup>+</sup>-K<sup>+</sup>-ATPase pumps.<sup>264,265,267,268</sup> Cocaine has some sympathomimetic activity but also causes seizures at higher doses.<sup>268</sup> As cocaine-induced hyperlactatemia is not observed experimentally in dogs treated with muscle relaxants, the increased muscle activity is believed to be more important than the adrenergic effects.<sup>268-271</sup> In a recent retrospective study on dogs presenting with presumptive cocaine toxicosis, 9/15 were hyperlactatemic defined as > 2.0 mmol/L with a median of 2.6 mmol/L (range 0.8 – 8.6 mmol/L)<sup>272</sup>

### *Propofol*

Propofol infusion syndrome (PRIS) refers to the development of acute metabolic acidosis, cardiac dysfunction, rhabdomyolysis, lipemia, and hepatomegaly or hepatic lipidosis in conjunction with propofol administration.<sup>273</sup> Hyperkalemia, lactic acidosis, and renal failure are also common features of this syndrome.<sup>274</sup> PRIS is most commonly seen in people receiving propofol for prolonged periods.<sup>275</sup> Impaired fatty acid oxidation and mitochondrial dysfunction are believed to be the major underlying pathophysiologic

mechanisms for PRIS.<sup>275,276</sup> To the authors' knowledge, PRIS has not yet been reported in dogs or cats.

### *Cyanide/Sodium Nitroprusside*

Cyanide toxicity occurs secondary to smoke inhalation, ingestion of toxins including stone fruit pits, cassava roots, bitter almonds and bamboo shoots, or infusions of sodium nitroprusside.<sup>30,55,277</sup> Cyanide inhibits aerobic metabolism by non-competitively inhibiting ferric iron in cytochrome c oxidase (complex IV), the final step in the electron transport chain.<sup>278</sup> This results in ATP depletion, acidosis, hyperlactatemia and venous hyperoxia due to impaired oxygen extraction.<sup>30,278,279</sup>

As sodium nitroprusside is metabolized into one molecule of nitric oxide and 5 molecules of cyanide, iatrogenic cyanide toxicity occasionally occurs.<sup>279,280</sup> Serial lactate measurements can be of value when monitoring for the development of cyanide toxicity secondary to sodium nitroprusside therapy.<sup>278,280,281</sup> Sodium nitroprusside also has the potential to cause Type A hyperlactatemia by inducing hypotension.

### *Glucocorticoids*

Glucocorticoids elicit hyperlactatemia by promoting amino acid conversion to pyruvate, inhibiting PDH, potentiating the hyperlactatemic effect of catecholamines and altering carbohydrate metabolism.<sup>282-288</sup> Both anti-inflammatory (1 mg/kg/day) and immunosuppressive (4 mg/kg/day) doses of prednisone resulted in statistically significant and clinically relevant increases in lactate concentrations in healthy Beagles.<sup>287</sup> In dogs receiving 4 mg/kg/day of methyl-prednisolone injected intramuscularly for 7 days, plasma lactate concentrations increased 8 fold.<sup>282</sup> In dogs with lymphoma, there is a possible association between hyperlactatemia and prednisone administration as 85% of dogs with lymphoma on prednisone were hyperlactatemic.<sup>134</sup>

### *Alcohols (Ethanol, Methanol, Propylene Glycol, Ethylene Glycol)*

Ethanol metabolism shifts the NAD<sup>+</sup>/NADH ratio in favor of lactate production.<sup>55,289,290</sup> The clinical relevance of ethanol induced hyperlactatemia remains controversial as it is mild and very uncommon in the absence of concurrent illness, such as decreased hepatic function, thiamine deficiency, seizure activity, or hypoperfusion.<sup>29,289-294</sup> Methanol is found in paint remover, windshield washing fluid, antifreeze and some illicit alcohols.<sup>55</sup> Alcohol dehydrogenase converts methanol into formaldehyde, which is subsequently converted into formate causing metabolic acidosis and inhibiting the electron transport chain promoting hyperlactatemia.<sup>55,295</sup>

Propylene glycol is a commonly used preservative, carbohydrate source in processed foods, chemical agent in coolants and cosmetics, and solvent for numerous drugs including but not limited to: etomidate, pentobarbital, phenobarbital, nitroglycerin,

silver sulfadiazine cream, lorazepam, esmolol, hydralazine, digoxin, trimethoprim-sulfamethoxazole, diazepam and some formulations of dexamethasone.<sup>245,296,297</sup> The reported LD<sub>50</sub> for oral consumption in dogs is 9.0 g/kg.<sup>296</sup> Although the toxic dose for intravenous administration has not been established in dogs, current recommendations in people are to avoid intravenous administration of greater than 40 mg/kg/hr or 1 g/kg/day.<sup>297,298</sup> Propylene glycol is metabolized first to lactaldehyde, and then into L-lactate, acetate and pyruvate or is converted to methylglyoxyl and then D-lactate.<sup>55,245,297,299,300</sup> Iatrogenic propylene glycol toxicity is well-documented in people treated with propylene glycol containing medications.<sup>297,299,301-304</sup> Although propylene glycol toxicity from drug administration has not been reported in companion animals, it may be under-recognized and should be considered in patients developing unexplained high anion gap metabolic acidosis while receiving propylene glycol containing medications. Propylene glycol toxicity with hyperlactatemia, confirmed by toxicological analysis on serum, has been reported in a dog following ingestion of an unknown toxin on a construction site.<sup>296</sup> Some formulations of activated charcoal contain propylene glycol along with the lactate precursor glycerol. The administration of 4 g/kg of activated charcoal containing propylene glycol has been experimentally associated with clinically significant but self-limiting hyperlactatemia (mean  $\pm$  SD 4.5  $\pm$  2.0 mmol/L) in healthy dogs.<sup>305</sup> Cats are also at risk for propylene glycol toxicity, developing erythrocyte Heinz bodies at comparatively lower doses than dogs due to their reduced glucuronidation capacity and clinically significant D-lactic acidosis at higher doses.<sup>300,306</sup>

Ethylene glycol, the highly palatable organic solvent found in common antifreeze, is metabolized into the toxic metabolites glycolic acid, oxalate and glyoxylic acid.<sup>55,245,307</sup> In this process, an increased NADH/NAD<sup>+</sup> ratio develops and pyruvate metabolism is inhibited thereby promoting lactate formation.<sup>307,308</sup> Ethylene glycol toxicity typically results in a mild to moderate increase in lactate concentration.<sup>307,309</sup>

### *Lactulose*

Lactulose is a synthetic, non-digestible disaccharide that is broken down into lactate and acetate in the colon.<sup>310</sup> If excessive quantities of lactulose are administered, or if the lactulose is retained in the colon, lactate can potentially be absorbed by the colonic mucosa resulting in systemic L and D-hyperlactatemia.<sup>311</sup> In healthy people administered lactulose, only modest elevations in plasma L and D-lactate are observed.<sup>312</sup> This has not yet been evaluated in dogs or cats.

### *Pesticides (Acetylcholinesterase Inhibitors and Strychnine):*

Hyperlactatemia from pesticide toxicity has been documented in dogs.<sup>313-315</sup> In a case series on dogs with aldicarb toxicity, a potent carbamate, 12/13 dogs presented with lactate > 2 mmol/L with a mean of 6.12 and a range of 1.86-10.06 mmol/L.<sup>313</sup> It is likely that hyperlactatemia in this patient group is multifactorial, resulting from muscle activity,

hypovolemia, and hypoxemia. Strychnine, an excitatory neurotoxin, results in extreme extensor muscle spasms, tetanic seizures and subsequent hyperlactatemia.<sup>315-318</sup>

### *Miscellaneous*

Other known drugs and toxins that have the potential to cause hyperlactatemia include: nucleoside HIV reverse transcriptase inhibitors,<sup>245</sup> 5-fluorouracil,<sup>319,320</sup> and the sugar alcohols fructose, sorbitol and xylitol.<sup>30,289,321-323</sup>

### **Type B3 Hyperlactatemia – Resulting from Congenital Errors in Metabolism**

Hyperlactatemia is linked to various congenital defects including: mitochondrial encephalomyopathy with lactic acidosis and stroke syndrome, defects in the pyruvate dehydrogenase complex or the TCA cycle, and defects in gluconeogenesis affecting pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase or glucose 6-phosphatase.<sup>55</sup> Mitochondrial myopathies have been reported in a German Shepherd Dog,<sup>324</sup> a Jack Russell Terrier,<sup>325</sup> Old English Sheepdogs,<sup>326,327</sup> Clumber Spaniels,<sup>328,329</sup> and Sussex Spaniels.<sup>329-331</sup>

### **D-Lactate**

Early literature suggested that mammals had limited capacity for D-lactate metabolism, but a number of more recent studies have documented D-lactate dehydrogenase in mammalian tissue and have shown that D-lactate is efficiently metabolized and reabsorbed by the kidney.<sup>332-337</sup> A small amount of D-lactate is normally produced through the glyoxalase pathway in mammalian species.<sup>22,23</sup> Increased bacterial fermentation in the gastrointestinal tract or altered carbohydrate metabolism can increase D-lactate production causing D-lactic acidosis.<sup>22,23,338</sup> Exogenous D-lactate may also be introduced via intravenous fluid solutions (such as racemic lactated Ringer's solution) or preservatives such as propylene glycol.<sup>55,306,339</sup>

In people, D-hyperlactatemia induced encephalopathy is recognized with short bowel syndrome,<sup>340-343</sup> and D-lactate is suspected to be an important contributor to the high anion gap seen in diabetic ketoacidosis.<sup>22</sup> Both D and L-lactate are readily produced in ruminant species during the fermentation of carbohydrates by ruminal flora<sup>335</sup> and encephalopathy from D-lactic acidosis is well documented due to grain overload, ruminal acidosis, calf diarrhea, drunken lamb syndrome and floppy kid disease.<sup>141,335,344-349</sup>

Elevated D-lactate concentrations have been documented in cats with diabetic ketoacidosis (mean  $\pm$  SD: 0.37  $\pm$  0.07 mmol/L)<sup>141</sup> propylene glycol intoxication (up to 7.1 mmol/L)<sup>306</sup> exocrine pancreatic insufficiency (17.7 mmol/L),<sup>350</sup> and gastrointestinal disease (median 0.36 mmol/L, range 0.04-8.33 mmol/L).<sup>351</sup> D-lactate should be considered a potential contributing factor in high anion gap metabolic acidosis.<sup>350</sup>

## CLINICAL APPLICATION

### Lactate as a Prognostic Indicator

#### *Human Medical Literature*

The association between admission hyperlactatemia and poor outcome has been demonstrated repeatedly across a range of patient populations in the human medical literature. Of 15,179 trauma patients, mortality was 22.7% in people presenting with lactate concentrations ([LAC])  $> 6$  mmol/L compared to just 2.26% in patients with [LAC]  $< 6$  mmol/L.<sup>352</sup> Similarly, in 5,360 people presenting to an emergency department (ED), 7-day mortality was 2.9% in patients with a [LAC]  $< 2$  mmol/L at admission, 7.8% for patients with [LAC] of 2-3.9 mmol/L, and 23.9% when admission [LAC] was 4.0 mmol/L or greater.<sup>353</sup> As a predictor of death, higher cut-offs for [LAC] are inherently associated with lower sensitivities but higher specificities while lower cut-offs are more sensitive but less specific. For example, in people presenting to the intensive care unit (ICU) or ED with hypotension, admission [LAC]  $> 6$  mmol/L,  $> 4$  mmol/L and  $> 2$  mmol/L had a sensitivity of 42%, 62%, and 88% and a specificity of 100%, 87% and 44% in predicting non-survival.<sup>354</sup> Nevertheless, a systematic review reported areas under the receiver operating characteristic curve (AUROC) for admission [LAC] as a marker of non-survival that ranged from poor to excellent (0.56 to 0.98).<sup>51</sup> Without accounting for study quality or weighting for study population size, the authors of the current review calculated a median AUROC of 0.71 from those data indicating moderate prognostic accuracy overall. Positive and negative predictive values also vary considerably between studies, an effect that is at least partially due to different mortalities in the populations studied. In the aforementioned systematic review, PPVs of admission [LAC] ranged from 4 to 97% (calculated median 33%) and NPVs ranged between 61 and 100% (calculated median 94%).<sup>51</sup>

Serial and calculated relative [LAC] measurements may provide more robust prognostic information than measurement at a single time point. In human patients with polytrauma, survival was 100% when [LAC] normalized within 24-hours of hospital admission.<sup>355</sup> Survival decreased to 78% if [LAC] remained increased for 48 hours following admission and to just 14% if [LAC] remained increased beyond 48 hours.<sup>355</sup> Similarly, mortality was 60% in patients with severe sepsis that demonstrated  $<10\%$  decrease in [LAC] within the first 6 hours of treatment compared to 19% in those with  $\geq 10\%$  decrease in [LAC] over the same time period.<sup>356</sup> Reported AUROC values for the ability of persistent hyperlactatemia to discriminate survivors from non-survivors range from 0.62 to 0.86 (calculated median 0.80).<sup>51</sup> Reported PPVs for persistent hyperlactatemia vary from 12-100% (calculated median 68%), and reported NPVs vary from 52-100% (calculated median 87%).<sup>51</sup> These results are comparable with the most well developed illness severity scoring systems (e.g. APACHE IV, MPM0-III, and SPS3) which are also reported to have highly variable AUROC values (median 0.81, range 0.62 to 0.92).<sup>357</sup>

Measurement of [LAC] appears to offer additional value as a marker of disease severity beyond traditional hemodynamic variables such as blood pressure and central venous oxygen saturation (ScvO<sub>2</sub>). In 28,150 patients with severe sepsis, normotensive patients presenting with [LAC] >4mmol/L were as likely to die (29.0%) as patients presenting with hypotension but with [LAC] < 4 mmol/l (29.3%).<sup>359</sup> This illustrates that high [LAC] can be a prognostic indicator even in the absence of clinically apparent hypotension. In the same study, mortality was 44.5% in patients with [LAC] >4 mmol/L and hypotension compared to 23.3% in normotensive patients with [LAC] <4mmol/L. People in septic shock who achieved a target of ScvO<sub>2</sub> ≥ 70% but not a ≥ 10% reduction in lactate per 2-hour period had a 41% mortality. (Puskarich 2012) In contrast, mortality was only 8% in those who achieved the lactate target but not the ScvO<sub>2</sub> target.<sup>359</sup>

In summary, the association between increased [LAC] and mortality is well established in the human medical literature. Overall the predictive power of [LAC] is moderate but varies from poor to excellent depending on the target population and the lactate measure used. In populations with relatively high mortality, [LAC] can effectively identify the group of patients at greater risk of non-survival, while in populations with low mortality, high [LAC] is associated with a high false positive rate and does not effectively predict outcome.<sup>353,360</sup> A low lactate concentration is a better predictor of survival than a high lactate is of death.

### *Veterinary Literature*

Presently, there are few highly powered, large-scale studies in the veterinary literature on which to base recommendations for using [LAC] measurements in patient evaluation and management. In an effort to provide some general guidelines, a literature search of PubMed, Scopus and CAB Abstracts performed for this review identified 34 clinical studies that investigated [LAC] as a prognostic indicator in dogs and cats.<sup>7,110,114,361-391</sup> As in the human medical literature,<sup>392</sup> there was marked heterogeneity in study sizes, study populations, mortality, and selected lactate cut-off points making comparison across studies challenging. Most, if not all, of the selected veterinary studies were hampered by relatively small sample sizes and, in many cases, the impact of euthanasia on prognostic variables was difficult or impossible to quantify.

Despite these limitations, the association between increased admission or initial [LAC] and mortality is well documented in dogs. From the available clinical studies that contained sufficient data for analysis, non-surviving dogs had significantly higher [LAC] than survivors in 18 of 22 studies.<sup>7,114,361-380</sup> Studies that found significantly higher [LAC] in non-survivors were in: dogs with blunt or penetrating trauma,<sup>261,362</sup> sepsis or SIRS,<sup>363-365</sup> spontaneous hemoabdomen,<sup>366</sup> IMHA,<sup>114</sup> Babesiosis,<sup>367-369</sup> heartworm,<sup>370</sup> general illness or injury,<sup>7,371</sup> GDV,<sup>372-375</sup> and following cholecystectomy.<sup>376</sup> A significant difference in [LAC] between survivors and non-survivors was not found in 4 of 22 studies: dogs with GDV,<sup>377</sup> blunt trauma,<sup>378</sup> Babesiosis<sup>379</sup> and soft tissue infections.<sup>380</sup>

In the 12 clinical studies in which mortality was reported, dogs with [LAC] above study-specific cut-offs always had a higher mortality than dogs with [LAC] below the cut-offs.<sup>110,114,364,367,368,372,373,375,377,383,384,385</sup> Across those studies, the difference in mortality ranged from 5 to 99% (calculated median 29%). Notably, [LAC] was a significant predictor of mortality in the Acute Patient Physiologic and Laboratory Evaluation (APPLE) scoring system which is a rigorous, veterinary, disease severity scoring system.<sup>381,382</sup>

Indicators of diagnostic performance (sensitivity, specificity, PPV, NPV and accuracy) for [LAC] measurements as a predictor of mortality in dogs were extracted or calculated from 14 studies (Tables 2, 3 and 4).<sup>110,114,362-364,367,368,372,373,375,377,383-385</sup> Population size across those 14 studies ranged from 20 to 173 dogs with mortality ranging between 4.5 and 71.6%. Performance of [LAC] was assessed for admission or initial samples, typically pre-surgical or prior to fluid resuscitation (Table 2), samples collected at a single, subsequent time point during hospitalization other than admission (Table 3), and relative [LAC] measurements (Table 4). Variance between studies was assessed by pooling the proportions of reported PPV, NPV and Accuracy by using the Freeman Tukey arcsine square root transformation method. A fixed-effects meta-analysis was then performed and weighted using the inverse of the variance<sup>393</sup> using commercially available software. (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX)

| Reference                           | Study Type;<br>Interest Group              | N   | Mortality<br>(%) | [LAC] Cut-off<br>(mmol/L) | Sensitivity and<br>Specificity (%)                               | Positive and<br>Negative Predictive<br>Values (%)                  | Accuracy<br>(%)                   |
|-------------------------------------|--------------------------------------------|-----|------------------|---------------------------|------------------------------------------------------------------|--------------------------------------------------------------------|-----------------------------------|
| De Papp<br>1999 <sup>110</sup>      | Retrospective; GDV                         | 101 | 13.9             | > 6.0                     | Sn: 92.9<br>Sp: 79.3                                             | PPV: 41.9<br>NPV: 98.6                                             | 81.2                              |
| Nel 2004 <sup>368</sup>             | Prospective;<br>babesiosis                 | 90  | 12.2             | > 2.5                     | Sn: 81.8<br>Sp: 54.4                                             | PPV: 20.0<br>NPV: 95.6                                             | 57.8                              |
| Jacobson<br>2005 <sup>367</sup>     | Prospective;<br>babesiosis                 | 20  | 25.0             | Increased*                | Sn: 80.0<br>Sp: 53.3                                             | PPV: 36.4<br>NPV: 88.9                                             | 60.0                              |
| Stevenson<br>2007 <sup>385</sup>    | Prospective;<br>systemically ill           | 80  | 12.5             | > 2.3                     | Sn: 60.0<br>Sp: 68.6                                             | PPV: 21.4<br>NPV: 92.3                                             | 67.5                              |
| Holahan<br>2010 <sup>114</sup>      | Retrospective;<br>IMHA                     | 173 | 23.1             | > 4.4<br>(ROC Analysis)   | Sn: 60.0<br>Sp: 76.7                                             | PPV: 43.6<br>NPV: 86.4                                             | 72.8                              |
| Zacher<br>2010 <sup>375</sup>       | Retrospective; GDV                         | 64  | 23.4             | ≥ 9.0                     | Sn: 73.3<br>Sp: 73.5                                             | PPV: 45.8<br>NPV: 90.0                                             | 73.4                              |
| Green<br>2011 <sup>111</sup>        | Retrospective; GDV                         | 84  | 11.9             | ≥ 4.1<br>(ROC Analysis)   | Sn: 90.9<br>Sp: 60.3                                             | PPV: 23.7<br>NPV: 97.8                                             | 63.9                              |
|                                     |                                            |     |                  | ≥ 6.0                     | Sn: 50.0<br>Sp: 74.3                                             | PPV: 20.8<br>NPV: 91.7                                             | 71.4                              |
| Conti-Patara<br>2012 <sup>363</sup> | Prospective; severe<br>sepsis/septic shock | 30  | 36.7             | ≥ 3.15                    | Sn: 63.6<br>Sp: 84.2                                             | PPV: 70.0<br>NPV: 80.0                                             | 76.7                              |
|                                     |                                            |     |                  | ≥ 3.30                    | Sn: 54.6<br>Sp: 89.5                                             | PPV: 75.0<br>NPV: 77.3                                             | 76.7                              |
| Green<br>2012 <sup>384</sup>        | Retrospective; GDV                         | 101 | 15.8             | ≥ 6.0                     | Sn: 62.5<br>Sp: 75.3                                             | PPV: 32.3<br>NPV: 91.4                                             | 73.2                              |
| Israeli<br>2012 <sup>377</sup>      | Prospective; GDV                           | 65  | 21.5             | Increased§                | Sn: 64.3<br>Sp: 43.1                                             | PPV: 23.7<br>NPV: 81.5                                             | 47.7                              |
|                                     |                                            |     |                  | > 6.0                     | Sn: 64.3<br>Sp: 66.7                                             | PPV: 34.6<br>NPV: 87.2                                             | 66.2                              |
|                                     |                                            |     |                  | > 7.2<br>(ROC Analysis)   | Sn: 57<br>Sp: 80                                                 | PPV: 44<br>NPV: 87                                                 | 75                                |
| Santoro Beer<br>2013 <sup>372</sup> | Retrospective; GDV                         | 73  | 10.9             | ≥ 7.4<br>(ROC Analysis)   | Sn: 75<br>Sp: 89                                                 | PPV: 46<br>NPV: 98                                                 | 88                                |
| Hall 2014 <sup>362</sup>            | Prospective;<br>trauma                     | 111 | 4.5              | ≥ 4.0<br>(ROC Analysis)   | Sn: 80<br>Sp: 56                                                 | PPV: 8<br>NPV: 98                                                  | 57                                |
| Cortellini<br>2015 <sup>364</sup>   | Retrospective;<br>septic peritonitis       | 81  | 35.8             | > 4.0                     | Sn: 37.9<br>Sp: 92.3                                             | PPV: 73.3<br>NPV: 72.7                                             | 72.8                              |
| Ateca 2015 <sup>383</sup>           | Retrospective;<br>critical illness         | 67  | 71.6             | ≥ 2.0                     | Sn: 64.6<br>Sp: 63.2                                             | PPV: 81.6<br>NPV: 41.3                                             | 64.2                              |
| <b>Overall<br/>(95% CI)</b>         |                                            |     |                  |                           | <b>Sn: 64.7<br/>(58.7 - 70.6)<br/>Sp: 71.7<br/>(69.0 - 74.4)</b> | <b>PPV: 35.0<br/>(30.7 - 39.4)<br/>NPV: 90.3<br/>(88.0 - 92.4)</b> | <b>69.8<br/>(67.3 -<br/>72.2)</b> |

\* Admission [LAC] greater than approximately 3.2 mmol/L (reference interval determined from 10 healthy dogs).

§ Admission [LAC] greater than 4.2 mmol/L which was the upper limit of reference interval determined in 79 healthy dogs

**Table 2:** Sensitivity (Sn), specificity (Sp), positive (PPV), negative (NPV) predictive values and accuracy for admission or initial lactate concentration [LAC] in predicting non-survival in dogs. The precise timing of the initial measurement of [LAC] was not always specified but occurred before surgery and/or the initiation of fluid therapy. Mortality includes both dogs that died and dogs that were euthanized; animals that were euthanized on the basis of financial constraints were not always excluded from analysis. Values were calculated from the available data by the authors of the current review if not provided in the study itself.<sup>5</sup>

| Reference                         | Study Type;<br>Interest<br>Group         | N    | Mortality* | [LAC] Cut-off<br>(mmol/L)              | Sensitivity and<br>Specificity (%)                               | Positive and<br>Negative<br>Predictive Values<br>(%)               | Accuracy<br>(%)               |
|-----------------------------------|------------------------------------------|------|------------|----------------------------------------|------------------------------------------------------------------|--------------------------------------------------------------------|-------------------------------|
| Nel 2004 <sup>368</sup>           | Prospective;<br>babesiosis               | 87   | 8 deaths   | > 2.5 at 8 hours                       | Sn: 87.5<br>Sp: 78.5                                             | PPV: 29.2<br>NPV: 98.4                                             | 79.3                          |
|                                   |                                          | 87   | 8 deaths   | > 5.0 at 8 hours                       | Sn: 50.0<br>Sp: 96.2                                             | PPV: 57.1<br>NPV: 95.0                                             | 92.0                          |
|                                   |                                          | 81   | 5 deaths   | > 2.5 at 16<br>hours                   | Sn: 100<br>Sp: 85.5                                              | PPV: 31.3<br>NPV: 100                                              | 86.4                          |
|                                   |                                          | 81   | 5 deaths   | > 5.0 at 16<br>hours                   | Sn: 100<br>Sp: 97.4                                              | PPV: 71.4<br>NPV: 100                                              | 97.5                          |
|                                   |                                          | 79   | 4 deaths   | > 2.5 at 24<br>hours                   | Sn: 100<br>Sp: 88.0                                              | PPV: 30.8<br>NPV: 100                                              | 88.6                          |
|                                   |                                          | 79   | 4 deaths   | > 5.0 at 24<br>hours                   | Sn: 75.0<br>Sp: 100                                              | PPV: 100<br>NPV: 98.7                                              | 98.7                          |
| Stevenson<br>2007 <sup>385</sup>  | Prospective;<br>systemically<br>ill      | 80   | 10 deaths  | > 2.3 at 6 hours                       | Sn: 60.0<br>Sp: 88.6                                             | PPV: 42.9<br>NPV: 93.9                                             | 85.0                          |
| Zacher <sup>375</sup><br>2010     | Retrospective<br>; GDV                   | 64¶  | 15 deaths  | ≥ 5.6 post-<br>resuscitation           | Sn: 80.0<br>Sp: 83.7                                             | PPV: 60.0<br>NPV: 93.2                                             | 82.8                          |
|                                   |                                          | 24§¶ | 11 deaths  | ≥ 6.4 post-<br>resuscitation           | Sn: 90.9<br>Sp: 76.9                                             | PPV: 76.9<br>NPV: 90.9                                             | 83.3                          |
| Cortellini<br>2015 <sup>364</sup> | Retrospective<br>; septic<br>peritonitis | 76   | 26 deaths  | > 2.5 post-<br>operatively             | Sn: 46.2<br>Sp: 88.0                                             | PPV: 66.7<br>NPV: 75.9                                             | 73.7                          |
|                                   |                                          | 24   | 13 deaths  | > 2.5 at 6 hours                       | Sn: 76.9<br>Sp: 100                                              | PPV: 100<br>NPV: 78.6                                              | 87.5                          |
|                                   |                                          | 55   | 19 deaths  | > 2.3 at 6 hours<br>(ROC analysis)     | Sn: 68<br>Sp: 92                                                 | PPV: 81<br>NPV: 84                                                 | 84                            |
|                                   |                                          | 54   | 22 deaths  | > 2.4 at 12<br>hours (ROC<br>analysis) | Sn: 54<br>Sp: 100                                                | PPV: 100<br>NPV: 76                                                | 81                            |
| <b>Overall<br/>(95% CI)</b>       |                                          |      |            |                                        | <b>Sn: 71.2<br/>(62.9 - 79.0)<br/>Sp: 92.3<br/>(90.1 - 94.3)</b> | <b>PPV: 62.5<br/>(54.6 - 70.1)<br/>NPV: 96.0<br/>(94.2 - 97.5)</b> | <b>87.8 (85.4<br/>- 90.0)</b> |

\*Number values indicate the number of deaths in the subset of animals evaluated.

§Subset-analysis of dogs with a high (> 9.0 mmol/L) presenting [LAC]

¶Precise timing of sample not specified but occurred post-resuscitation

**Table 3:** Sensitivity (Sn), specificity (Sp), positive (PPV), negative (NPV) predictive values and accuracy for lactate concentration [LAC] measured at a single, subsequent time point other than admission in predicting non-survival in dogs. Mortality includes both dogs that died and dogs that were euthanized; animals that were euthanized on the basis of financial constraints were not always excluded from analysis. Values were calculated from the available data by the authors of the current review if not provided in the study itself.<sup>5</sup>

| Reference                         | Study Type;<br>Interest Group           | N    | Mortality* | [LAC] Cut-off<br>(mmol/L)                                | Sensitivity<br>and<br>Specificity<br>(%)                         | Positive and<br>Negative<br>Predictive Values<br>(%)               | Accuracy<br>(%)               |
|-----------------------------------|-----------------------------------------|------|------------|----------------------------------------------------------|------------------------------------------------------------------|--------------------------------------------------------------------|-------------------------------|
| Nel 2004 <sup>368</sup>           | Prospective;<br>babesiosis              | 43   | 7 deaths   | Decrease in [LAC]<br>≤ 50% at 8 hours                    | Sn: 71.4<br>Sp: 83.3                                             | PPV: 45.5<br>NPV: 93.8                                             | 81.4                          |
|                                   |                                         | 38   | 4 deaths   | Decrease in [LAC]<br>≤ 50% at 16 hours                   | Sn: 75.0<br>Sp: 79.4                                             | PPV: 30.0<br>NPV: 96.4                                             | 78.9                          |
| Stevenson<br>2007 <sup>385</sup>  | Prospective;<br>systemically ill        | 28   | 6 deaths   | Decrease in [LAC]<br>< 50% at 6 hours                    | Sn: 83.3<br>Sp: 68.1                                             | PPV: 41.7<br>NPV: 93.8                                             | 71.4                          |
| Zacher<br>2010 <sup>375</sup>     | Retrospective;<br>GDV                   | 64¶  | 15 deaths  | Decrease in [LAC]<br>≤ 42.2%<br>(ROC Analysis)           | Sn: 100<br>Sp: 61.2                                              | PPV: 44.1<br>NPV: 100                                              | 70.3                          |
|                                   |                                         | 24§¶ | 11 deaths  | Δ[LAC] ≤ 4.0<br>mmol/L<br>(ROC Analysis)                 | Sn: 81.8<br>Sp: 92.3                                             | PPV: 90.0<br>NPV: 85.7                                             | 87.5                          |
|                                   |                                         | 24§¶ | 11 deaths  | Decrease in [LAC]<br>≤ 42.5%<br>(ROC Analysis)           | Sn: 100<br>Sp: 84.6                                              | PPV: 84.6<br>NPV: 100                                              | 91.7                          |
| Cortellini<br>2015 <sup>364</sup> | Retrospective;<br>septic<br>peritonitis | 18   | 12 deaths  | Persistent ↑[LAC]<br>post-op<br>(> 2.5 mmol/L)           | Sn: 91.7<br>Sp: 100                                              | PPV: 100<br>NPV: 85.7                                              | 94.4                          |
|                                   |                                         | 20   | -          | Decrease in [LAC]<br>≤ 21% at 6 hours<br>(ROC Analysis)  | Sn: 54£<br>Sp: 91£                                               | -                                                                  | -                             |
|                                   |                                         | 18   | -          | Decrease in [LAC]<br>≤ 42% at 12 hours<br>(ROC Analysis) | Sn: 82£<br>Sp: 100£                                              | -                                                                  | -                             |
| <b>Overall<br/>(95% CI)</b>       |                                         |      |            |                                                          | <b>Sn: 93.3<br/>(84.2 - 99.1)<br/>Sp: 77.3<br/>(70.3 - 83.6)</b> | <b>PPV: 61.3<br/>(51.0 - 71.1)<br/>NPV: 96.8<br/>(92.3 - 99.6)</b> | <b>80.3 (74.8<br/>- 85.3)</b> |

\*Number values indicate the number of deaths in the subset of animals evaluated.

§Subset-analysis of dogs with a high (> 9.0 mmol/L) presenting [LAC]

¶Precise timing of second sample not specified but occurred post-resuscitation

£Values not included in calculation of overall test performance as insufficient data available

**Table 4:** Sensitivity (Sn), specificity (Sp), positive (PPV), negative (NPV) predictive values and accuracy for relative [LAC] measurements in predicting non-survival in dogs. Mortality includes both dogs that died and dogs that were euthanized; animals that were euthanized on the basis of financial constraints were not always excluded from analysis. Values were calculated from the available data by the authors of the current review if not provided in the study itself.<sup>5</sup>

Depending on the cut-off value used and timing of sample collection, sensitivity, specificity, PPV and NPV varied considerably between studies. Nevertheless, these data convincingly demonstrate consistently high NPVs compared to PPVs (i.e. that low lactate predicts survival much better than high lactate predicts death). In those studies in which it was reported, the AUROC for initial and admission [LAC] to correctly predict outcome ranged from 0.66 to 0.89 (calculated median 0.71). For single subsequent and relative [LAC] measurements, the AUROC for predicting outcome ranged between 0.66 and 0.98 (calculated median 0.86). Using accuracy as an overall measure of predictive performance, [LAC] had a moderate prognostic ability. Measurement of [LAC] at a single, subsequent time point appeared to outperform [LAC] measured at admission and relative measurements (overall accuracy: 87.8%, 69.8% and 80.3%, respectively). Determining the ideal timing of subsequent sample collection and the respective ideal cut-off value requires further studies on specific diseases and conditions. It is important to emphasize that, because no effort was made to account for qualitative differences between studies, readers are cautioned in over-applying these data to their own clinical populations. Nevertheless, dogs with persistent hyperlactatemia appear to be at greater risk of death.

Few clinical studies have evaluated [LAC] as a prognostic indicator in cats and the results differ. In 102 cats requiring intravenous fluid therapy, an admission [LAC] > 4.0 mmol/L was associated with lower survival (17/27: 63% vs 20/22: 91%) and increased median duration of hospitalization (48 hrs, Range 3-123 vs 24 hrs, Range 8-183) compared to eulactatemic cats.<sup>386</sup> Cats with [LAC] > 4.0 mmol/L and a decrease of  $\geq 30\%$  within 8 hours of admission had a higher survival (9/10: 90%) than those where [LAC] decreased < 30% (1/6: 17%). In 111 cats presenting to an ED,<sup>387</sup> 123 sick cats hospitalized for emergency care,<sup>388</sup> and in 51 cats with septic peritonitis,<sup>389</sup> [LAC] was not significantly different between non-survivors and survivors. In contrast, in 26 cats with septic peritonitis<sup>390</sup> and in 600 cats admitted to an ICU, non-survivors had significantly higher [LAC] compared to survivors.<sup>381</sup> In the latter study, [LAC] could discriminate between the two groups with an AUROC of 0.70, indicating moderate prognostic accuracy.<sup>394</sup> Furthermore, in 39 hypotensive cats admitted to an intensive care unit, cats with normal initial [LAC] had a 66% mortality rate compared to 92.6% in those with [LAC]  $\geq 2.5$  mmol/L (overall mortality 85%).<sup>391</sup>

Overall, it appears that the association between increased [LAC] and mortality in cats may be less consistent than in dogs and people. One plausible explanation is that lactate might increase exponentially in cats with increasing severity of disease rather than in a linear fashion. Hence, cats with mild to moderate compromise may exhibit only mild increases in lactate such that more cats with marginally increased [LAC] would die compared to other species. Additional studies are required to further characterize how lactate increases in cats with worsening disease severity and further elucidate its prognostic value.

When using [LAC] as a prognostic indicator in individual clinical cases, recommendations to the owner should be couched in relatively conservative terms (i.e. for

patients with severe hyperlactatemia it may be appropriate to say “complications are more likely”). In agreement with the human medical literature, overall, there is good evidence to suggest that a normal [LAC] is a better predictor of survival than a high [LAC] is of death. Dogs with normal [LAC] at admission are highly likely to survive as demonstrated by the generally high NPVs reported by most studies. While higher [LAC] can indicate a poorer prognosis, an increased [LAC] at any time during hospitalization should never be used as sole justification to withhold treatment or recommend euthanasia. As an example, the mortality in dogs with GDV presenting with moderately or markedly increased [LAC] was 13 to 57% higher than in dogs presenting with normal to mildly increased [LAC].<sup>110,372,373,375,377,384</sup> However, 79% of dogs with [LAC]  $\geq$  4.1 mmol/L,<sup>373</sup> 65% of dogs with [LAC]  $\geq$  5.9 mmol/L,<sup>377</sup> and 58% of dogs with [LAC]  $\geq$  6.0 mmol/L<sup>110</sup> survived with treatment. Furthermore, 54% of dogs with admission [LAC]  $\geq$  9.0 survived to discharge following treatment.<sup>375</sup> In the future, even when ideal cut-offs have been identified for specific conditions, the clinician will still never know whether an individual patient is in the, let's say, 5% of patients that will survive or the 95% that will die. Further research is required before firmer recommendations can be made about the predictive value of lactate as a marker of mortality in cats.

Lactate compares well with other predictors of outcome or measures of disease severity in veterinary medicine including those requiring more input variables and that are more cumbersome to use. As previously mentioned, reported AUROCs for [LAC] as a predictor of mortality in dogs range from 0.66 to 0.98. Reported AUROCs for the veterinary survival prediction index 2 (SPI2) that requires 7 variables, range from 0.61-0.77.<sup>395-397</sup> Reported AUROCs for the APPLE score vary between 0.78 and 0.93 for the 10-variable score<sup>382,397,398</sup> and 0.85 to 0.95 for the 5-variable score.<sup>381,399</sup> Finally, the AUROC was 0.91 for the 6-variable animal trauma triage score (ATT).<sup>362,400</sup> Irrespective of the current evidence base, in all species, hyperlactatemia warrants increased suspicion for severe underlying disease, a comprehensive diagnostic approach, and increased time and resource allocation, particularly when it persists despite treatment.

## **Lactate as a Therapeutic Guide**

### *Human Medical Literature:*

Oxygen derived variables are considered important indices of tissue blood flow and oxygen utilization.<sup>401-404</sup> As such, several of these variables, including [LAC], have been investigated as therapeutic guides and indicators of the restoration of tissue blood flow. Human ICU patients prospectively randomized to receive therapy targeting a decrease in [LAC] by  $\geq$  20% per 2-hour period had a mortality of 33.9%, while mortality in the standard care group was 43.5%.<sup>405</sup> Although this difference was not statistically significant in the initial analysis, after adjusting for risk factors, in hospital mortality was significantly lower in the group targeting a reduction in [LAC] with a hazard ratio of 0.61 (95% CI, 0.43-0.87). Patients in the [LAC] targeted group also had lower Sequential Organ Failure Assessment

(SOFA) scores, required less inotropic and ventilator support, and were discharged earlier than the control group.<sup>405</sup> In human patients with severe sepsis or septic shock and an initial lactate  $\geq 4.0$  mmol/L, using mandated serial lactate monitoring to guide therapy significantly reduced 28-day mortality compared to measurement of only initial [LAC] (23.5% vs 39.6%).<sup>406</sup> The group in which [LAC] was serially measured also had lower 24-hour SOFA scores, and shorter hospitalization, mechanical ventilation and vasopressor dependency times.<sup>406</sup> Similarly, patients with septic shock subsequent to pneumonia were randomly allocated to receive standard early-goal directed therapy (EGDT), EGDT targeting a [LAC] reduction of 10%, or EGDT targeting a [LAC] reduction of 30%.<sup>407</sup> Illness severity scores, 28-day mortality and length of ICU stay were significantly reduced in the 2 groups targeting [LAC] reduction with the 30% [LAC] reduction group demonstrating the greatest benefit.<sup>407</sup> In a meta-analysis that included four randomized controlled trials, inclusion of [LAC] reduction in the treatment of septic patients significantly reduced the risk of death (relative risk ratio 0.65, 95% CI, 0.49-0.85).<sup>408</sup> In contrast, other studies have documented reduced patient morbidity without significantly different mortality between patient groups.<sup>409,410</sup> Human patients with sepsis and hypoperfusion resuscitated with a protocol targeting either a [LAC] reduction of 10% or  $\text{ScvO}_2 \geq 70\%$  in addition to CVP and MAP had similar mortality, indicating non-inferiority between the two strategies.<sup>411,412</sup> This suggests that [LAC] could be effectively used to guide resuscitation in septic patients when more expensive or invasive monitoring techniques are either not available or inappropriate. Inclusion of targeted [LAC] reduction to the Surviving Sepsis Campaign bundle decreased mortality from 24.5% to 17.9% in patients with severe sepsis or septic shock who achieved the therapeutic targets.<sup>413</sup> Lactate normalization was subsequently added to the 2012 Surviving Sepsis Campaign guidelines<sup>414</sup> and has remained a part of the recently updated 2016 guidelines.<sup>415</sup>

The human medical literature supports [LAC] reduction as a useful, non-invasive resuscitation marker in critically ill patients. Treatment protocols incorporating [LAC] reduction have been associated with lower morbidity and mortality but the ideal target for lactate reduction remains unknown.<sup>416</sup> In some studies, administered treatments<sup>411</sup> and rate of lactate reduction<sup>405</sup> were similar between treatment groups despite outcome benefits in those receiving lactate targeted therapy. This may suggest that lactate targeted therapy may positively influence patient outcomes by promoting increased clinician concern, more frequent patient re-evaluation, and perhaps more individualized resuscitation efforts.

### *Veterinary Literature*

Experimental trials in animals have also demonstrated that normalization of traditional hemodynamic parameters including heart rate and blood pressure do not necessarily guarantee adequate oxygen delivery at the capillary level.<sup>417-419</sup> In a recent prospective, observational trial including 30 dogs in hypovolemic or septic shock, central venous [LAC] remained  $\geq 2$  mmol/L in 7/30 (23%) of patients and  $\text{ScvO}_2$  remained  $< 70\%$  in

11/30 (38%) of patients despite normalization of traditional hemodynamic parameters including heart rate and blood pressure.<sup>420</sup> These results highlight the importance of using multiple clinical end points to assess the efficacy of shock resuscitation.

Although there is evidence that lactate-guided therapy is associated with improved outcomes in some patient groups in the human medical literature, there are currently insufficient data to make specific recommendations as to how and when lactate-guided therapy should be implemented in veterinary medicine. The use of [LAC] measurement as a component of a protocolized therapeutic strategy has been prospectively evaluated in 30 dogs that met the criteria for severe sepsis or septic shock due to pyometra, but conclusions regarding the superiority of including [LAC] measurements could not be made from this report as a control group was not included.<sup>363</sup>

Given that persistent hyperlactatemia has been consistently associated with increased mortality in veterinary patients, it seems reasonable to suggest that persistent hyperlactatemia should trigger careful re-evaluation of the patient. Hyperlactatemia secondary to simple, volume responsive hypoperfusion should rapidly resolve with restoration of circulating volume. As the half-life of lactate in healthy animals is estimated to be 20-60 minutes<sup>83,87,89,91</sup> it seems reasonable to expect lactate concentrations to decrease by 50% every 1-2 hours and to expect resolution of hyperlactatemia within 6-12 hours. When hyperlactatemia is persistent or decreasing more slowly than anticipated, volume status should be reassessed and treated accordingly. If the patient is deemed volume replete, interventions should be directed at other ways to optimize oxygen delivery as clinically indicated (e.g., transfusion therapy, oxygen support, inotropes). Once efforts to improve oxygen delivery have been exhausted, causes of Type B hyperlactatemia should be considered.

## **Measurement of Lactate in Other Body Fluids**

### *Peritoneal Effusion*

Studies in both human and veterinary medicine have evaluated peritoneal fluid [LAC] to differentiate septic from aseptic and benign from malignant abdominal disease.<sup>109,423-427</sup> In people, normal [LAC] in abdominal effusions cannot be reliably used to exclude septic or surgical abdominal disease.<sup>421,422</sup> Further, although an increase in peritoneal [LAC] supports the presence of intra-abdominal pathology, peritoneal [LAC] alone cannot definitively discriminate between sepsis and malignancy.<sup>423</sup>

Experimentally, peritoneal fluid [LAC] increases markedly and rapidly following intestinal strangulation in dogs but does not increase with non-ischemic bowel obstructions.<sup>424,425</sup> Generally, dogs and cats with septic peritonitis have higher abdominal fluid [LAC] than those without but there is considerable overlap between groups.<sup>426,427</sup> In one small study (n=19), peritoneal fluid [LAC] > 2.5 mmol/L was 100% sensitive and 91% specific identifying septic peritonitis in dogs<sup>427</sup> and a difference of > 2.0 mmol/L between

abdominal fluid and blood [LAC] had a sensitivity of 63% and a specificity of 100%.<sup>427</sup> The apparently high sensitivities and specificities from this study are very likely biased because of the low subject numbers detecting a lower number of false positives and false negatives and so should be used with great caution. The same study reported findings from 18 cats: abdominal fluid [LAC] > 2.5 mmol/L was 67% sensitive, 67% specific and 67% accurate in detecting septic peritonitis and a difference of > 0.5 mmol/L between blood and peritoneal fluid [LAC] was 78% sensitive, 78% specific and 78% accurate.<sup>427</sup>

As both leukocytes<sup>68,427</sup> and neoplastic cells<sup>428-430</sup> are known to produce lactate, it is possible that aseptic but highly inflammatory or neoplastic effusions may also be associated with increased peritoneal [LAC]. Hence, abdominal fluid [LAC] may not be able to reliably differentiate abdominal sepsis from other highly inflammatory abdominal processes such as severe pancreatitis or neoplastic disease. Further, the presence of abdominal drains is associated with a high peritoneal fluid [LAC]<sup>431,432</sup> so the absolute peritoneal fluid [LAC] and PFL:BL ratio should be considered unreliable in the diagnosis of abdominal sepsis when using samples collected from abdominal drains.<sup>431,432</sup>

### *Synovial Fluid*

Several studies in people have reported significantly higher synovial fluid [LAC] in people with non-gonococcal septic arthritis, seropositive rheumatoid arthritis, and microcrystal-induced arthropathies compared to seronegative rheumatoid arthritis, degenerative or gonococcal arthritis with variable overlap between groups.<sup>433-436</sup>

Normal synovial fluid [LAC] in dogs has been reported as  $2.8 \pm 0.6$  mmol/L (mean  $\pm$  SD, n=17)<sup>437</sup> and  $1.7 \pm 0.44$  mmol/L (mean  $\pm$  SD, n=6).<sup>438</sup> Mean synovial fluid [LAC] was significantly higher in dogs with septic arthritis (mean  $\pm$  SD,  $8.9 \pm 2.1$  mmol/L, n=8) compared to normal dogs (mean  $\pm$  SD,  $2.8 \pm 0.6$ , n=17), dogs with osteoarthritis (mean  $\pm$  SD,  $3.2 \pm 1.3$ , n=30) or immune-mediated inflammatory arthritis (mean  $\pm$  SD,  $2.8 \pm 0.6$  mmol/L, n=19).<sup>437</sup> Synovial fluid lactate was not correlated with nucleated cell count, and [LAC]  $\geq 6.5$  mmol/L was able to discriminate patients with septic arthritis with 100% sensitivity (95% CI 63%-100%) and 100% specificity (95% CI 95%-100%).<sup>437</sup> There was also no significant difference detected in synovial fluid lactate concentration between 6 dogs with osteoarthritis secondary to spontaneous cranial cruciate rupture (mean  $\pm$  SD,  $2.3 \pm 0.85$  mmol/L) and 6 normal dogs ( $1.7 \pm 0.44$  mmol/L).<sup>438</sup>

These preliminary data suggest that a high synovial fluid [LAC] in dogs might help differentiate septic from immune-mediated arthritis and osteoarthritis which may be particularly helpful given that joint cultures are often unreliable in the diagnosis of septic arthritis in dogs.<sup>439</sup> Albeit uncommon, the impact of erosive arthritis (rheumatoid arthritis) on synovial fluid [LAC] is presently unknown and is a potential confounder. To the authors' knowledge, the utility of synovial fluid lactate in cats has not been investigated.

## *Cerebrospinal Fluid*

Bacterial production of lactate is believed to account for only 10% of CSF [LAC] in cases of bacterial meningitis, with the majority originating from neurons, glial cells and leukocytes.<sup>440-442</sup> In people, brain hypoxia and vascular compromise (i.e. intracranial hemorrhage, mass lesions, trauma, stroke, seizures, hypoglycemic coma) also increase CSF [LAC].<sup>443,444</sup> When confounding conditions have been excluded, CSF [LAC] can differentiate septic from aseptic meningitis.<sup>440,441,445,446</sup> In a meta-analysis including 33 studies and 1885 people, the measurement of CSF [LAC] had a pooled sensitivity of 93% (95% CI 0.89-0.96) and specificity of 96% (95% CI 0.93-0.98) in discriminating septic from aseptic meningitis using a cut-off of 3.9 mmol/L.<sup>447</sup> Similarly, in a meta-analysis that included 25 studies and 1692 people but excluded those with CNS disease that could increase CSF [LAC] (e.g., stroke, seizures, cerebral hypoxia, brain trauma), CSF [LAC] had a pooled sensitivity of 96% (95% CI, 0.95-0.98), specificity of 94% (95% CI, 0.93-0.96) and AUROC of 0.984 in differentiating septic meningitis from aseptic meningitis.<sup>444</sup> Consequently, empirical antimicrobial therapy is recommended in post-operative neurosurgical patients if CSF [LAC] is  $\geq 4.0$  mmol/L.<sup>442,448</sup> CSF lactate cannot be used to rule out bacterial meningitis in patients previously treated with antibiotics.<sup>447</sup>

Healthy cats have CSF [LAC] of  $1.16 \pm 0.06$ <sup>449</sup> and in healthy dogs CSF [LAC] has been reported as 1.1-2.0 mmol/L,<sup>450</sup> 0.42-1.8 mmol/L,<sup>451</sup> and  $1.9 \pm 0.61$  mmol/L (mean  $\pm$  SD).<sup>452</sup> Increased CSF lactate has been documented in dogs following experimental intracerebral and subarachnoid hemorrhage<sup>453,454</sup> and in cats following experimental spinal cord trauma and brain injury.<sup>160,455</sup> CSF [LAC] does not effectively differentiate dogs with intervertebral disc herniation from those without, nor is it predictive of outcome.<sup>456</sup> CSF [LAC] is correlated with plasma [LAC] in dogs with intracranial disease and CSF [LAC] is higher in dogs with worse neurological scores, however, CSF [LAC] could not accurately identify dogs with intracranial disease.<sup>450</sup> To the authors' knowledge, there are no clinical investigations into CSF lactate as a marker of septic meningitis in dogs or cats.

## *Pericardial Effusion*

[LAC] has been measured in the pericardial fluid of dogs in an effort to distinguish neoplastic from non-neoplastic effusions. Median pericardial fluid [LAC] was significantly higher in dogs with neoplasia (9.2 mmol/L, range 1.9-12.5 mmol/L, n = 28) but overlapped with values obtained from dogs with non-neoplastic effusions (3.7 mmol/L, range 1.3-12.7 mmol/L, n = 13).<sup>457</sup> The difference between peripheral blood [LAC] and pericardial fluid [LAC] was also not useful, in part because 61% of all dogs with pericardial effusion were hyperlactatemic. To our knowledge, [LAC] in pericardial effusions have not been evaluated in cats.

## *Dystocia*

In people, fetal distress during parturition can be identified by measuring [LAC] from fetal scalp or umbilical cord blood.<sup>458,459</sup> Umbilical vein [LAC] in 70 puppies from 17 parturitions was higher in pups delivered vaginally compared to pups delivered via elective C-section.<sup>460</sup> Further, umbilical vein [LAC] was higher in pups that died within 48 hours (mean  $\pm$  SD,  $12.2 \pm 6.7$  mmol/L) when compared to surviving pups ( $6.55 \pm 3.3$  mmol/L). Mean  $\pm$  SD umbilical [LAC] in “severely stressed” pups (low APGAR (Appearance, Pulse, Grimace, Activity, Respiration) score) at birth was  $8.6 \pm 5.2$  mmol/L compared to  $7.5 \pm 3.6$  mmol/L in those with a medium APGAR score and  $4.6 \pm 1.4$  mmol/L in healthy pups (high APGAR score). For comparison, jugular lactate from healthy, 4-day old pups is  $3.83 \pm 1.38$  (mean  $\pm$  SD) mmol/L.<sup>461</sup> [LAC] measured from the amniotic sacs of 95 puppies<sup>462</sup> was highest for stillborn pups, lower for pups born via vaginal delivery and lowest in pups born via elective C-section; however, a significant difference between surviving pups and those that died within 48 hours was not detected.<sup>462</sup>

## **CLINICAL MANAGEMENT OF HYPERLACTATEMIA**

When faced with a patient with hyperlactatemia, it is important to remember that an increase in [LAC] may be an indicator of underlying disease but is not, in and of itself, harmful. If the cause of hyperlactatemia is physiologic, such as exercise or a brief, uncomplicated epileptic seizure, treatment to correct the hyperlactatemia is not indicated. In these patients, [LAC] should decrease quickly following cessation of muscle activity, e.g. decreasing by 50% per hour or normalizing within 1-2 hours.<sup>83,87,89,91</sup> Conversely, if the hyperlactatemia is a consequence of a pathologic process, therapy should primarily be directed at the underlying cause. Although the origins of hyperlactatemia in shock are likely multifactorial, the most common cause of hyperlactatemia in veterinary emergency practice is, almost certainly, tissue hypoperfusion. Regardless of the underlying cellular mechanisms, restoration of tissue perfusion typically results in a rapid and profound decrease in [LAC] in patients with hypoperfusion-induced hyperlactatemia. Persistently increased [LAC] following apparent optimization of oxygen delivery or hyperlactatemia without evidence of impaired tissue perfusion raises concern for occult shock or causes of Type B hyperlactatemia. Although these suggestions are logical and not particularly contentious, further research is necessary to definitively support them.

Interpreting [LAC] in conjunction with other markers of tissue metabolism may help to unravel the origins of hyperlactatemia in specific patients. For example, in the absence of increased muscle activity, increased [LAC] in conjunction with low venous oxygen content or saturation and/or high venous carbon dioxide tension suggests inadequate tissue oxygen delivery (via macrocirculatory compromise, microcirculatory compromise, and rarely anemia or hypoxia).<sup>463-468</sup> Alternatively, an increased lactate concentration in conjunction with an increased peripheral venous oxygen concentration may suggest reduced oxygen extraction (for example due to mitochondrial dysfunction with impaired

oxygen utilization such as in SIRS) or microcirculatory shunting.<sup>469,470</sup> Epinephrine-induced upregulation of Na<sup>+</sup>-K<sup>+</sup>-ATPase and extramitochondrial glycolysis may be an under recognized cause of hypokalemia and should be considered when faced with hyperlactatemia with concurrent hypokalemia.<sup>208,471</sup>

Macrocirculatory derangements should be treated with intravenous fluid therapy, blood products, supplemental oxygen, vasopressors and inotropic support as appropriate.<sup>472</sup> The ideal strategies to diagnose and treat microcirculatory and mitochondrial derangements have not been defined. Although not readily available for use in clinical practice, microcirculatory monitoring techniques such as sidestream microscopy are presently under investigation<sup>473-475</sup> and therapies to improve microcirculatory flow such as dobutamine, nitrates and calcium sensitizers are also being evaluated.<sup>476,477</sup> Research is also ongoing into interventions targeting mitochondrial dysfunction including cofactor supplementation, mitochondrial anti-oxidants, reactive oxygen species scavengers and mitochondrial membrane stabilizers.<sup>478-481</sup> The ideal therapy for microcirculatory and mitochondrial dysfunction remains elusive with conflicting data from both experimental and clinical trials.<sup>482,483</sup> Cofactor supplementation with thiamine is a safe and rational adjunctive treatment for hyperlactatemia given that thiamine pyrophosphate is a cofactor for pyruvate metabolism. Discontinuation of drugs that might promote hyperlactatemia (e.g., epinephrine, corticosteroids) should be considered if clinically appropriate when managing patients with unexplained hyperlactatemia to help distinguish between persistent hypoperfusion and Type B2 hyperlactatemia. In some patients, unexplained hyperlactatemia might indicate malignancy (among other Type B causes) and supports the need for further diagnostics and specific therapy.

Based on the evidence available to date, specific pharmacologic intervention to accelerate lactate metabolism or inhibit lactate production is not recommended and may be harmful.<sup>484-489</sup> Although a number of pharmacologic agents including dichloroacetate (a pyruvate dehydrogenase activator),<sup>486-492</sup> ouabain (an Na<sup>+</sup>-K<sup>+</sup>-ATPase inhibitor),<sup>200,201,296</sup> and  $\beta$ -adrenergic antagonists<sup>209,296</sup> effectively reduce [LAC], reductions in mortality have been inconsistent. Indeed, some studies have documented increased organ dysfunction and higher mortality associated with these interventions.<sup>486-489</sup> This may be because lactate is an important alternative cellular energy source, especially for tissues such as the myocardium and brain during periods of stress.<sup>9,27,201,209</sup>

The use of buffers such as sodium bicarbonate to treat the acidosis that often accompanies hyperlactatemia is also controversial and potentially harmful. Sodium bicarbonate has the potential to cause hyponatremia, hyperosmolality, ionized hypocalcemia, overshoot alkalosis, and hypercarbia, as well as intracellular and CSF acidosis.<sup>493-499</sup> Sodium bicarbonate can also promote lactate production by upregulating the glycolytic enzyme phosphofructokinase.<sup>29,500-504</sup> A number of experimental animal studies have documented bicarbonate therapy to be of no benefit or detrimental in the management of hyperlactatemia,<sup>500,501</sup> and the administration of bicarbonate in people with hyperlactatemia is associated with increased mortality without significant

improvement in hemodynamic variables.<sup>505-508</sup> Other alkalinizing therapies that have been investigated include tris(hydroxymethyl)aminomethane (THAM), bicarb, dialysis, and selective sodium-hydrogen exchanger 1 inhibitors<sup>484,497,508,509</sup> with the latter potentially showing some early promise for the treatment of hypovolaemic shock.<sup>509</sup>

## CONCLUSION

Lactate is an important energy storage molecule, the production of which preserves cellular energy production and mitigates the acidosis from ATP hydrolysis. Hyperlactatemia ultimately reflects a disruption in cellular bioenergetics of physiologic or pathophysiologic origin. Understanding the different etiologies of hyperlactatemia is helpful in determining 1) whether the presence of hyperlactatemia is of clinical concern and 2) how to best direct treatment. The human medical and veterinary literature demonstrates that increased [LAC] is associated with increased morbidity and mortality. Overall, in populations with sufficiently high mortality, increased lactate is moderately predictive in identifying non-survivors. Serial lactate measurements and relative measures appear to outperform single measurements. Importantly, a normal [LAC] predicts survival better than a high one predicts death. Consequently, lactate is a valuable triage and risk stratification tool that can be used to separate patients into higher and lower risk categories, but hyperlactatemia does not justify withholding resources or performing euthanasia because a significant number of patients with increased [LAC] will survive with therapy. The use of lactate as a therapeutic guide has shown promising results in people but data are sparse in veterinary species. Increased [LAC] in body fluids other than blood should raise the index of suspicion for septic or malignant processes. Hyperlactatemia is a protective response so treatment of hyperlactatemia should be directed at the underlying cause.

## **CHAPTER 2: PRE-ANALYTICAL, ANALYTICAL AND POST-ANALYTICAL CONSIDERATIONS FOR PLASMA LACTATE MEASUREMENT IN THE DOG**

### **INTRODUCTION**

It is prudent to consider pre-analytical, analytical and post-analytical influences whenever ordering, implementing and interpreting the results of a diagnostic test. Important considerations include sample type, sample handling, collection tubes, collection technique, sample site, analyzer methodology, and potential interferents. Lastly, analyzer and species-specific reference intervals must be established for reliable interpretation and application of results.

### **PRE-ANALYTICAL CONSIDERATIONS**

#### **Sample Type**

Lactate concentration can be measured in whole blood, plasma or serum. The term whole blood lactate refers to the combined concentration of intraerythrocytic and plasma lactate fractions following either red blood cell lysis or the use of conversion formulae.<sup>56,385,510</sup> Many lactate analyzers used in clinical practice use whole blood as the preferred test sample, but do not cause erythrocyte lysis and therefore actually report plasma lactate concentrations. Others measure plasma lactate and then use conversion factors and algorithms, generally based on human data, to display whole blood lactate.<sup>512,513</sup>

Lactate transport in canine red blood cells occurs at a rate 1.5-3 times faster than in people, 1.5-17 times faster than horses, and more than 50 times faster than in several ruminant species.<sup>56,59,61</sup> As lactate transport into canine and human red blood cells remains constant over a range of plasma lactate concentrations, plasma lactate can be used to estimate whole blood lactate with known packed cell volumes.<sup>56</sup> This is not the case for all species. In horses, erythrocyte lactate transport kinetics demonstrate marked interindividual variation, such that plasma lactate cannot reliably be used to estimate whole blood lactate concentrations.<sup>56</sup> In people, intraerythrocytic lactate concentrations are approximately 50% that of plasma at equilibrium, such that whole blood lactate is roughly 70% that of plasma lactate.<sup>64,510,514,515</sup> In dogs, intraerythrocytic lactate concentrations equilibrate at approximately 70% that of plasma within 5 minutes of incubation.<sup>56</sup> As such, we can anticipate whole blood lactate in dogs to be roughly 90% that of plasma, assuming an average packed cell volume of 45%. Lactate transport kinetics in feline erythrocytes have yet to be investigated, but as red blood cell lysis does not appear to affect lactate concentration, intracellular and extracellular lactate concentrations are believed to be similar.<sup>142</sup>

Although sample type should be selected based on manufacturer specifications, one must remain aware of what is reported by the analyzer to a) ensure an appropriate reference interval is used in practice and b) ensure results are comparable when consulting the scientific literature.

### **Sample Handling and Collection Tubes**

Sample handling guidelines for lactate measurement vary depending on whether the sample will be measured immediately or after a delay. Red blood cells, white blood cells and platelets consume glucose and produce lactate so a delay in the separation of serum and plasma from the cellular fraction can cause a progressive decrease in glucose and a progressive increase in lactate unless a glycolytic inhibitor is used. The preferred method of analysis is to collect the sample into a heparinized syringe, capillary tube or blood tube and process it as soon as possible, preferably within 5 minutes.<sup>516,517</sup> The use of commercially available blood gas syringes or capillary tubes containing dry, lyophilized heparin are considered ideal, as manually heparinized syringes have been associated with dilutional errors in lactate measurement.<sup>518</sup> Commercial lithium heparin collection tubes can also be used.<sup>517</sup> At room temperature, lactate concentrations in heparinized whole blood will increase by 0.2-0.5 mmol/L per 30 minutes,<sup>142,519,520</sup> but should only rise by 0.2 mmol/L after 120 minutes if stored in an ice bath.<sup>520,521</sup> In contrast, a recent study showed that lactate concentrations in heparinized canine whole blood increase by >20% within 15 minutes when stored at room temperature, and within 25 or 40 minutes for anaerobic and aerobic samples, respectively, when refrigerated.<sup>516</sup>

Serum lactate concentrations will also significantly increase in the time required for blood to clot in a serum collection tube.<sup>142</sup> Lactate concentrations measured in serum samples from cats have been shown to increase by 0.4 to 1.2 mmol/L in the time required to allow blood to clot,<sup>142</sup> and a similar effect has been documented in people.<sup>519</sup> At room temperature, on-the-clot serum lactate increased by 0.98 mmol/L in the first hour, while samples kept at 4 degrees Celsius increased by 0.5 mmol/L.<sup>142</sup>

Collection tubes with sodium fluoride, a glycolytic inhibitor, and potassium oxalate, an anticoagulant, are preferred for samples from dogs and cats being sent to an external lab.<sup>142,520,522</sup> Plasma lactate in sodium fluoride/potassium oxalate did not significantly increase when stored at either 25 degrees Celsius or 4 degrees Celsius for up to 8 hours.<sup>142,520</sup> Plasma must be promptly separated because although sodium fluoride will arrest ongoing lactate production, lactate will continue to be taken up by erythrocytes which may impact results.<sup>56</sup> Lastly, the anticoagulant sodium citrate interferes with lactate measurement and therefore should be avoided.<sup>523,524</sup> Importantly, these are general suggestions and sample processing should be based on recommendations specific to the analyzer or reference laboratory being used.

## Collection Technique

Sampling technique is an important consideration when measuring blood lactate. Struggling has a variable effect on plasma lactate concentrations likely dependent upon the degree of muscle activity. In one study in cats, a 5-minute spray bath test resulted in a rapid and dramatic increase in blood lactate concentration (mean  $\approx 7.0$  mmol/L).<sup>77</sup> In contrast, in another small study in cats, mild to moderate struggling associated with restraint for venipuncture in cats did not result in hyperlactatemia.<sup>78</sup> Although prolonged occlusion of a vessel, such as with a tourniquet, can result in increased lactate concentrations,<sup>525</sup> transient occlusion for routine blood sampling does not have a significant effect.<sup>526</sup> Nevertheless, patient struggling and extended venous stasis should be avoided when drawing blood samples for lactate analysis. When sampling from an intravenous catheter, inadvertent contamination with intravenous fluids can affect results and should be avoided.<sup>2,3,527</sup> The push-pull method, whereby a volume equal to 300% of the catheter dead space is aspirated into a syringe and reinfused without disconnecting the syringe before a second syringe is used to aspirate the sample has been shown to be equivalent to direct venipuncture in dogs undergoing anesthesia.<sup>528</sup>

## Sample Site

Blood samples for lactate measurement can be collected from peripheral venous, central venous, or arterial vessels as well as peripheral capillaries. In people, differences between arterial and central venous or peripheral venous lactate concentrations are small when lactate concentrations are normal to mildly increased.<sup>529-534</sup> The gradient between sample sites can significantly increase in the face of hypoperfusion.<sup>533-539</sup> While arterial lactate concentrations provide a holistic view of total body lactate metabolism, peripheral concentrations reflect the metabolic state of the specific tissue being drained by the particular vessel being sampled. As such, venous lactate may be more sensitive than arterial lactate in detecting tissue hypoperfusion.<sup>538</sup>

Investigations into the reliability of capillary blood lactate have produced conflicting results.<sup>510,538,540-544</sup> In theory, capillary lactate might better reflect oxygenation at the tissue level than vascular sampling. "Milking" of a pin prick site for a capillary sample, however, may lead to a dilutional effect due to inclusion of interstitial fluid in the sample.<sup>545</sup> In addition, the term "capillary sample" may be misleading, as a lancet prick likely results in blood originating from arterioles and venules in addition to capillaries.<sup>546</sup>

In a population of 60 healthy dogs, small but significant differences in lactate concentration were identified between samples from the cephalic vein ( $1.57 \pm 0.47$  mmol/L), jugular vein ( $1.25 \pm 0.49$  mmol/L) and femoral artery ( $1.43 \pm 0.52$  mmol/L).<sup>547</sup> Similarly, in 18 stable dogs undergoing orthopedic or soft tissue surgery, peripheral arterial and venous lactate concentrations were almost identical, and lingual arterial samples varied only slightly with limits of agreement of  $-0.3$  to  $0.3$  mmol/L.<sup>546</sup> In a study on healthy cats, samples from the medial saphenous and jugular veins were similarly comparable.<sup>78</sup>

Research investigating different lactate sampling sites in ill or injured companion animals is scarce. In 53 dogs referred to a university veterinary hospital, poor correlation was found between jugular venous and capillary (pinnal) lactate concentrations (correlation coefficient 0.58, mean difference 0.72 mmol/L, limits of agreement -1.55 to 2.99 mmol/L).<sup>548</sup> In an experimental study, a significant difference was detected between lactate measured in the femoral artery or pulmonary vein and the portal vein.<sup>549</sup> These results highlight the importance of sample site specific reference intervals and confirm that different sample sites cannot be used interchangeably when trending lactate concentrations in a patient.

In clinical practice, peripheral venous, central venous or arterial samples can be used for lactate measurement, but ideally the same site should be used for serial evaluations.

## **ANALYTICAL CONSIDERATIONS**

### **Analyzers**

Importantly, classification schemes for biochemical analyzers differ across sources. Many references differentiate analyzers based on their primary location of use, referring to “portable,” “hand-held,” “point-of-care,” “benchtop,” and “laboratory” analyzers.<sup>2,3,511,512,550,551</sup> In contrast, for the purpose of method validation, the terms “definitive,” “reference” and “field” analyzer are preferred.<sup>552</sup> Definitive methods represent the ideal gold standard test. Definitive methods are well researched, highly stable, often technically demanding and costly (e.g. mass spectrophotometry).<sup>552</sup> Depending on the analyte of interest, however, definitive methods may be of limited availability.<sup>552</sup> The term reference method is applied to high-quality analyzers that have been validated through method comparison studies with definitive analyzers or through the use of certified reference materials.<sup>552</sup> Lastly, field methods are generally cost effective, user friendly analyzers that are validated by comparison with a reference method or another field method.<sup>552</sup> Veterinary diagnostic laboratories generally use either reference or field methods, whereas point-of-care testing is generally limited to field methods.<sup>552</sup>

A range of hand-held point-of-care, benchtop point-of-care, and benchtop laboratory lactate analyzers are now available. As expected, these analyzers vary with respect to methods, reported results, and reference intervals. The most common analytical methods for measurement of lactate are enzymatic amperometry and enzymatic colorimetry via spectrophotometry.<sup>553</sup> Spectrophotometry is the quantitative analysis of molecules via light absorption, while amperometry is the measurement of ions in solution based on an electrical current.<sup>554</sup> Older laboratory analyzers such as the Spectronic Genesys 5 (Fisher Scientific, Pittsburgh, PA, USA)<sup>142</sup> and the Konelab 30i (Thermo Electron Corp, Vantaa, Finland)<sup>522</sup> enzymatically oxidize lactate to produce NADH, which is then

detected via spectrophotometry and correlated to sample lactate concentrations. Most newer bench-top analyzers, such as the Stat Profile Critical Care Xpress (Nova Biomedical, Waltham, MA), the YSI 1500 (Yellow Springs Instruments Co., Yellow Springs, OH), the Radiometer ABL800 (Radiometer Medical, Copenhagen, Denmark), the RAPIDPoint 500 (Siemens Healthcare, Medfield, MA), Rapidlab 865 (Bayer Diagnostics, East Walpole, MA) and the Roche Cobas Integra (Roche Diagnostics, Indianapolis, IN) use enzyme amperometry to measure lactate concentrations. Most of these analyzers allow plasma from a whole blood sample to pass through a semipermeable membrane covering an electrode. The membrane contains lactate oxidase that reacts with lactate in the sample to form hydrogen peroxide. The hydrogen peroxide is subsequently oxidized by the electrode creating an electrochemical current proportional to lactate concentration.<sup>551,553</sup> Some benchtop analyzers such as the Roche Cobas Integra, link the lactate oxidase to a peroxidase reaction with a dye-producing reagent such as 4-aminoantipyrine, which is then quantified colorimetrically.

Several hand-held lactatometers are also readily available for clinical use. In the Lactate Pro (Arkray Inc, Kyoto, Japan) and Lactate Scout (SensLab, Leipzig, Germany) whole blood reacts with lactate oxidase producing hydrogen peroxide and pyruvate. The hydrogen peroxide then reacts with potassium ferricyanide to produce an anodic current, which is proportional to the lactate concentration on the sample.<sup>551</sup> The iSTAT (Abbott Laboratories, Abbott Park, IL) uses a similar technology, but the hydrogen peroxide reacts with platinum instead of potassium ferricyanide.<sup>551</sup> The Accutrend (Roche Diagnostics, Basel, Switzerland) uses the lactate oxidase reaction to create a colour change proportional to the concentration of lactate in the sample, which is then quantified colorimetrically by reflectance photometry.<sup>551,513</sup>

The enzymes used in both amperometric and spectrophotometric measurement of L-lactate are stereospecific. These analyzers cannot, therefore be used to measure D-lactate. D-lactate can be effectively measured by unique methods including: D-lactate-specific enzymatic techniques (i.e. D-lactate dehydrogenase), gas chromatography, high-performance liquid chromatography or capillary electrophoresis.<sup>555</sup> The majority of these methods require specialized laboratories and personnel, but automated methods of D-lactate measurement are becoming increasingly available.<sup>556,557</sup>

A number of studies have been performed examining the analytic performance of handheld analyzers in dogs using different analyzers as reference methods. The lactate pro appears to slightly underestimate the Nova statprofile M (Nova Biomedical, Waltham, MA) with a mean bias of -0.07 mmol/L.<sup>558</sup> The Lactate Pro more significantly underestimated values measured by the Roche/Hitachi 917 (Roche Diagnostics, Indianapolis, Indiana), with average difference of 0.748 mmol/L, becoming more significant at higher lactate concentrations.<sup>559</sup> The i-STAT and Lactate Pro both showed a reasonably high level of agreement with the Stat Profile Critical Care Xpress in dogs and cats, while the Accutrend and Lactate Scout demonstrated less agreement.<sup>551,560</sup> In another study, the Accutrend correlated poorly with the Rapidlab 865 due to a significant positive bias.<sup>511</sup> In this study,

values above 5 mmol/L were outside of the 95% limits of agreement suggesting inadequate accuracy at these levels.<sup>511</sup> Similarly, when the Lactate Scout was compared to the Konelab 30i, the level of agreement was good below 5 mmol/L but above 5 mmol/L the Lactate Scout reported lower concentrations than the Konelab 30i.<sup>522</sup> The same was true when the Lactate Plus (Nova Biomedical, Waltham, MA) was compared to the GEM Premier 3000 (Instrumentation Laboratory, Bedford, MA)<sup>554</sup> Lastly, when the Istat and Accutrend were compared against the YSI 1500, the Accutrend showed poorer agreement than the Istat.<sup>513</sup> The Accutrend is no longer being manufactured.<sup>553</sup>

Although these studies provide useful data to the veterinary literature, some common shortcomings were identified. Several of the studies lack sufficient sample representation at the high end of the measurable range.<sup>511,522,551,559,512</sup> This is not surprising for data collected from clinical populations. Healthy dogs typically have lactate concentrations less than 3 mmol/L, and only very ill or injured dogs have lactate concentrations greater than 5 mmol/L. In addition, a number of studies compared analyzers reporting whole blood lactate against analyzers reporting plasma lactate.<sup>511,522,551,559,554</sup> These comparisons are complicated by the fact that intraerythrocyte lactate concentrations differ from plasma lactate concentrations, as previously described. Some analyzers claim to measure whole blood lactate but do not lyse erythrocytes and as such actually measure plasma lactate, while others measure plasma lactate but use algorithms based on human blood to report whole blood lactate concentrations. These differences have the potential to impact results and are of particular relevance when establishing reference intervals or performing method comparison studies.

Surprisingly, upon reviewing the literature, it is apparent that validation studies evaluating the commonly used and referenced benchtop analyzers for lactate measurement in dogs are lacking.<sup>553</sup> This may be largely because a “gold standard” for lactate measurement is not well defined.<sup>513,521,554,561</sup> As “validation is essential in order to demonstrate that an analytical test is appropriate for its intended purpose,”<sup>562</sup> however, it is prudent that this void in the literature be addressed.

## **Interference**

Rapid infusion of lactated Ringer’s solution (LRS) has the potential to affect plasma lactate concentrations. Lactate concentrations remained within the reference interval in healthy adult dogs treated with LRS at 2 ml/kg/hr for 12 hours, and at 10 ml/kg/hr for 1 hour.<sup>563</sup> In an experimental hemorrhage model in dogs comparing resuscitation with a 20 ml/kg bolus of LRS over 10-12 minutes to hypertonic saline, lactate concentrations did not significantly differ between groups.<sup>564</sup> Administration of LRS at 4 ml/kg/hr for 6 hours in dogs with lymphoma with normal baseline lactate concentrations resulted in a mild to moderate increase in blood lactate compared to controls in the first hour, but concentrations returned to baseline by the second hour of infusion.<sup>565</sup> These studies used racemic LRS with an equal mixture of L and D-lactate totaling 28 mmol/L.<sup>566</sup> Recent evidence has shown that D-lactate may have deleterious systemic effects<sup>566,567</sup> and as such,

non-racemic LRS containing only the L-lactate isomer has become available. Infusion of non-racemic L-LRS containing 28 mmol/L of L-lactate at a rate of 180 ml/kg/hr in 6 healthy Beagles resulted in blood lactate concentrations that were significantly higher at 60 minutes compared to dogs treated with 0.9% NaCl (mean±SD of approximately 3.2±0.6 mmol/L and 1.4±0.6 mmol/L respectively).<sup>566</sup> The average increase in lactate concentration from T0 to T60 was 1.7±1.1 mmol/L, with a maximum reported lactate concentration of 6.4 mmol/L. Hyperlactatemia resolved within 60 minutes of discontinuing fluids in all patients.<sup>566</sup>

Ethylene glycol is another well-recognized interferent. Several point-of-care analyzers using lactate oxidase amperometry erroneously measure the ethylene glycol metabolites glycolic acid and glyoxalic acid as lactate falsely reporting severe hyperlactatemia.<sup>307,568-570</sup> Analyzers that use lactate oxidase spectrophotometry do not appear to experience the same interference.<sup>307</sup> As the false positive result does not occur on all analyzers, the use of a “lactate gap” between different analyzers has been suggested as a potential diagnostic technique to rapidly identify patients with ethylene glycol toxicosis.<sup>307-309,569</sup> In a report on two dogs with ethylene glycol toxicity, one analyzer falsely reported plasma lactate concentrations as > 30 mmol/L.<sup>570</sup>

In one case report describing a person with a profoundly elevated D-lactate concentration (110 mmol/L) positive interference by D-lactate was suspected to be the cause of erroneously reported L-hyperlactatemia.<sup>339,571</sup> Presently, there is no evidence to suggest D-lactate interference is of significant clinical concern except potentially in the rarest of scenarios associated with profound D-hyperlactatemia. As previously mentioned, sodium citrate is also another interferent and therefore sodium citrated anticoagulated collection tubes should be avoided.<sup>523,524</sup>

Other reported interferents include bromide,<sup>513</sup> hemoglobin based oxygen carriers,<sup>513</sup> and thiocyanic acid.<sup>584</sup> Lastly, increased PCV has been associated with falsely decreased lactate in horses,<sup>513</sup> and people,<sup>584</sup> but has not yet been investigated in dogs.

## **POST-ANALYTICAL CONSIDERATIONS**

### **Reference Intervals**

Reference intervals for lactate have been established in dogs on several benchtop and handheld analyzers.<sup>7,139,461,511,547,558,559,572,573</sup> Reference intervals vary between analyzers, but the majority of studies demonstrate an upper limit varying between 2.5 and 3.0 mmol/L.

Puppies up to 4 weeks of age have been reported to have higher normal lactate concentrations than adult dogs.<sup>461</sup> The authors postulated that the age-related hyperlactatemia was a result of post birth ischemia/reperfusion injury or an alternative fuel

source in the face of limited capacity for gluconeogenesis. Although struggling was deemed to be minimal, the authors also commented that perhaps mild struggling influenced the results more than anticipated.<sup>461</sup>

| <b>Analyzer</b>          | <b>n</b>        | <b>Reference Interval</b>                                                                             | <b>Reference</b>              |
|--------------------------|-----------------|-------------------------------------------------------------------------------------------------------|-------------------------------|
| Nova Biomedical          | 60              | 0.3 - 2.5 mmol/L                                                                                      | Hughes 1999 <sup>547</sup>    |
| Nova Stat Profile M      | 37<br>135<br>30 | 1.07 – 6.59 mmol/L; up to 4 days<br>0.80 – 4.60 mmol/L; from 10-28 days<br>0.12 – 3.44 mmol/L; adults | McMichael 2005 <sup>461</sup> |
| Nova Stat Profile M      | 30              | 0.4 – 2.6 mmol/L                                                                                      | Thorneloe 2007 <sup>558</sup> |
| Lactate Pro              | 28              | ≤ 0.8 – 2.9 mmol/L                                                                                    | Thorneloe 2007 <sup>558</sup> |
| Lactate Pro              | 60              | ≤ 0.8 – 3.3 mmol/L                                                                                    | Tas 2008 <sup>559</sup>       |
| Accutrend                | 100             | 1.2 – 3.1 mmol/L                                                                                      | Stevenson 2007 <sup>511</sup> |
| Rapidlab 865             | 100             | 0.46 – 2.31 mmol/L                                                                                    | Stevenson 2007 <sup>511</sup> |
| YSI Select Model 2700    | 20              | 0.73 – 2.90 mmol/L                                                                                    | Lagutchik 1998 <sup>7</sup>   |
| Manual Spectrophotometry | 17              | 0.6 – 1.3 mmol/L                                                                                      | Durocher 2008 <sup>139</sup>  |
| RAPIDPoint 500           | 51              | 0.43 – 2.10 mmol/L                                                                                    | Bachmann 2018 <sup>517</sup>  |

Table 5 – Previously reported reference intervals for whole blood or plasma lactate in dogs

## CONCLUSION

Upon consideration of the available literature, it is apparent that consistency is of paramount importance when measuring and interpreting lactate concentrations. Lactate can be measured in whole blood or plasma and from different sample sites, however the same sample type and sample site should be used when evaluating serial lactate concentrations in an individual patient. Ideally, samples should be collected using atraumatic technique into heparinized blood gas syringes or heparinized collection tubes and processed immediately. If a significant delay in measurement is anticipated, the sample should be refrigerated and/or a glycolytic inhibitor should be used. Practitioners should be conscientious of potential interferents and should apply analyzer and species specific reference intervals whenever possible. Lastly, it has become apparent that, despite widespread clinical use, benchtop lactate analyzers have not been adequately validated in dogs. Additional investigations are warranted.

## **CHAPTER 3: METHODS OF ANALYZER VALIDATION**

### **INTRODUCTION**

Before a new diagnostic method is introduced to clinical practice, it must be validated for use. Method validation is performed to demonstrate that a method is suited to its intended analytical purpose.<sup>562,574,575</sup> The complete validation process involves a three-staged, sequential evaluation of analytical performance, diagnostic performance and patient outcome performance.<sup>552</sup>

Analytical performance ensures a method operates within manufacturer specifications and pre-determined quality requirements by evaluating measures such as accuracy and precision.<sup>552</sup> As analytical performance varies between species, species-specific testing is recommended.<sup>552</sup> Diagnostic performance evaluates the discriminatory capacity of a method via measures such as sensitivity, specificity, and predictive values.<sup>552</sup> Studies of diagnostic performance should not be performed until acceptable analytical performance has been demonstrated and appropriate reference intervals have been established.<sup>552</sup> Once analytical and diagnostic performance have been characterized, patient outcome studies can be performed to evaluate the impact of a method in clinical practice by considering variables such as cost, turnaround time, and effects on patient morbidity or mortality.<sup>552</sup>

As described in Chapter 1, a comprehensive literature review revealed numerous publications evaluating the diagnostic performance of lactate in small animal veterinary emergency and critical care medicine. Chapter 2 described a handful of studies assessing the analytical performance of small, hand-held lactate analyzers. Surprisingly, we were unable to identify any publications validating the benchtop methods used to validate the hand-held devices, despite their widespread use in clinical and laboratory practice.<sup>553</sup>

This chapter is intended to provide a review of the method validation process and a summary of previously performed method validation studies for the measurement of lactate in dogs.

### **EVALUATION OF ANALYTICAL PERFORMANCE**

The term “method validation study” refers to a series of tests performed to ensure a particular method’s analytical characteristics satisfy predetermined quality requirements.<sup>552</sup> A method validation study is different to and more rigorous than a method verification study, which ensures an analyzer satisfies manufacturer claims following installation.<sup>552</sup> Importantly, method validation is distinct from internal quality control. The former is carried out once and determines what can be expected from the

method, whereas the latter is performed regularly and is used to monitor performance over time.<sup>575</sup>

Accuracy, precision, linearity, range, lower limit of quantification, interference, parallelism, and stability are all potential components of biomarker method validation.<sup>576</sup> A method validation study must include, at minimum, an evaluation of accuracy (bias) and precision as these parameters are used to establish total analytical error which can then be compared to pre-specified quality requirements.<sup>552</sup>

Precision is a measure of repeatability or reproduceability. Precision is evaluated via replication studies and presented as either coefficient of variation or standard deviation. Imprecision in a method indicates the presence of random error. Both intra-assay (within day, repeatability) and inter-assay (between day, reproduceability) precision can be evaluated. Intra-assay precision is evaluated over less than 24 hours under consistent conditions by the same operator.<sup>577,578</sup> Inter-assay precision is evaluated over a minimum of 20 days, during different shifts, and with different operators.<sup>578</sup> Standard solutions, commercial controls or pooled fresh patient samples are considered suitable test materials.<sup>578</sup> At least 20 replicates over 2 or 3 levels are recommended.<sup>578</sup> If Gaussian distribution cannot be confirmed after exclusion of outliers, transformation may be required.<sup>578</sup> Mean, standard deviation and coefficient of variation should be determined,<sup>578</sup> whereby:

$$CV(\%) = (SD / \text{mean}) \times 100\%$$

If the SD or CV exceeds total allowable error ( $TE_a$ ) prespecified by the ASVCP based on clinical consensus<sup>579</sup> or other nominated clinical decision limit, the method should be rejected.<sup>578</sup> Alternatively, if available, quality requirements based on biological variability can be applied to gauge acceptability.<sup>578-580</sup> For example, a CV limit of 13.6% has been recommended for lactate by the Spanish Society of Clinical Chemistry and Molecular Biology (SEQC).<sup>580</sup>

Accuracy (bias, systematic error) reflects the difference between the test value and the “true value.” Inaccuracy occurs due to the presence of constant bias and/or proportional bias. Bias can be determined by the following formula:

$$\text{Bias}(\%) = (\text{Mean}_{\text{ref}} - \text{Mean}_{\text{test}}) / \text{Mean}_{\text{ref}} \text{ (Harr 2013)}$$

Constant bias suggests that the test method produces results that are consistently above or below the true value across the tested range.<sup>577</sup> Constant bias is reflected by the deviation of the y-intercept from zero in regression testing or by the mean difference being consistently different than zero on a Bland-Altman plot.<sup>578</sup> Proportional bias indicates that the magnitude of error changes with analyte concentration across the measured range (e.g. may be greater at higher concentrations).<sup>552,577,578</sup> Proportional bias is reflected by

deviation of the slope of the regression line from zero or by an increase in scattering of data points from the zero line at higher concentrations on a Bland-Altman plot.<sup>578</sup>

Most commonly, the “true value” is determined via comparison to a gold-standard laboratory method, known as a “definitive method.”<sup>552</sup> Oftentimes, these tests are only available at specialized laboratories and are costly or technically demanding, such as mass spectrophotometry.<sup>552</sup> The “definitive method” is then used to validate more accessible and user friendly bench-top methods typically found in diagnostic laboratories, labelled “reference methods.”<sup>552</sup> “Field methods” are point-of-care tests which are able to be subsequently validated via comparison to a “reference method.”<sup>552</sup> Evaluating the analytical performance of bench-top analyzers for the measurement of lactate is complicated by the fact that a gold-standard, definitive method has not been well-defined.<sup>521,561</sup> An alternative approach in such circumstances is to use certified reference material to represent the “true value.”<sup>552,575,577</sup> If this approach is used, commutability and matrix effects on the test system must be considered.<sup>552</sup>

Quality requirements for accuracy can be based on either biological variability data<sup>579,580</sup> or on clinical consensus.<sup>578,579</sup> A % bias limit of 8% has been recommended for lactate by the SEQC based on biological variability. The ASVCP does not specify a consensus-based quality requirement for accuracy via method comparison but instead suggests calculating observed total error ( $TE_{obs}$ ) using % bias and % CV and then applying specified  $TE_a$  limits.<sup>552</sup> The ASVCP does, however, indicate that % Bias should be less than  $TE_a$  as a quality requirement for spike and recovery analysis.<sup>578</sup>

Quality requirements, against which the overall analytical performance of a test method is compared, may be based on biologic variation data, regulatory requirements or expert opinion.<sup>552</sup> The American Society of Veterinary Clinical Pathologists (ASVCP) encourages the use of consensus-based allowable total error ( $TE_a$ ) as a suitable analytical quality requirement for assessment of overall analytical performance.<sup>552</sup> When  $TE_a$  are based on clinician consensus, they are selected to reflect the greatest total error possible without resulting in laboratory variation that could affect clinical decision making.<sup>579,581</sup>  $TE_a$  reflects the amount of random and systematic error that can be tolerated for a single result.<sup>578</sup> Alternatively, other nominated limits such as performance requirements specified by the Clinical and Laboratory Improvement Amendments (CLIA) can be applied.<sup>552,579</sup> If available,  $TE_a$  based on biological variation data can be used to gauge acceptability.<sup>579,580</sup> These limits, however, may be too stringent for certain analytes measured with veterinary diagnostic instruments, in which case consensus-based limits are preferred.<sup>579,580</sup> If the observed total error ( $TE_{obs}$ ) of the method is less than the  $TE_a$  or CLIA performance requirement, the method is considered acceptable.  $TE_{obs}$  is calculated using the imprecision and bias of the test method with one of the following formulae:

$$TE_{obs} = \text{Bias}(\%) + 2 \text{ CV}(\%)^{552,579}$$

$$TE_{obs} = \text{Bias (test units)} + 3 \text{ SD (test units)}^{578,579}$$

Where % Bias is the mean of the differences between test method results and true results, and CV/SD are determined from precision analysis.<sup>578</sup> Published TE<sub>a</sub> requirements vary substantively between references. For example, for lactate, the ASVCP recommends a consensus-based TE<sub>a</sub> limit of 40%,<sup>579</sup> whereas the CLIA recommends consensus-based limits of 10 or 30%,<sup>579</sup> and the SEQC advises biological variation-based limits of 30.4 or 39.7%.<sup>580</sup> Further research in this arena is clearly required to develop a more consistent and standardized approach.

A number of other properties may also be investigated to more thoroughly examine the analytical characteristics of a method during validation. Linearity is evaluated by plotting the mean value of replicates measured at each concentration against the expected value and then visually examining the plot to determine the range in which the assay is linear.<sup>578</sup> The terms analytical or reportable range refer to the range of concentrations that are reliable, satisfying pre-determined bias and precision requirements.<sup>578</sup> Linear range is the part of the analytical range in which measured and true values have a linear relationship.<sup>578</sup> In addition, several detection limits can be investigated: limit of blank reflects the highest value that may be observed from a blank sample, limit of detection reflects the lowest value that can be detected, and limit of quantification reflects the lowest concentration that can be detected with satisfactory precision and accuracy.<sup>578</sup> Interference studies can also be performed to evaluate whether potential interferents (i.e. hemoglobin, bilirubin, heparin) cause systematic error.<sup>578</sup> Other investigations that can be performed include stability testing and equivalency analysis via method comparison.<sup>578</sup>

Comparison of two methods answers a variety of important questions, such as: Are the methods considered comparable with respect to analytic validity? Does a method-specific reference interval need to be established? Can the same clinical decision thresholds be used for both analyzers? Can results from the two methods be directly compared when trending serial measures in one patient over time?<sup>552</sup>

Method comparison studies are generally performed to determine inaccuracy (bias) of the new method relative to a definitive or validated reference method, with established accuracy.<sup>552,578</sup> In this situation, any bias is assumed to be due to inaccuracy of the test method.<sup>552</sup> If neither method has been sufficiently validated to be considered a definitive or reference method, then the bias identified in the comparison study will include analytical error from both methods.<sup>552</sup> It is recommended that samples span the entire reportable range including adequate sample numbers at range limits.<sup>578</sup> When two quantitative methods using continuous numerical data are compared, such as plasma lactate concentration, they should be analyzed using regression and Bland-Altman difference plot analysis.<sup>552</sup> The results should be plotted on a scatter plot with the reference method on the x-axis.<sup>578</sup>

Determining the correlation coefficient (r) is a measure of association but is not a measure of agreement.<sup>577,578</sup> It is performed, rather, to allow determination of the

appropriate type of regression analysis.<sup>577,578</sup> If  $r$  is  $\geq 0.99$  (for data with a wide range) or  $> 0.975$  (for data with a narrow range) then linear regression can be used to estimate bias at a decision making point using the following equation:

$$Y_c = a + bX_c \text{ (a = y intercept, b = slope)}^{578}$$

$$\text{Bias} = Y_c - X_c^{578}$$

( $X_c$  = specific, clinically important concentration)

If correlation is insufficient, the data may need to be improved either via additional replicates to decrease variance, gathering additional data, or using other types of regression analysis (i.e. Deming, Passing-Bablok).<sup>577</sup>

Furthermore, two major assumptions in ordinary least squares regression linear regression are 1) that there is no error in the reference method and 2) any error in the new method is constant across the range and normally distributed.<sup>577,578</sup> Because these assumptions are rarely met in practice, Passing-Bablok or Deming regression may be preferable.<sup>577,578</sup> Passing-Bablok regression allows for the inclusion of extreme values and variable imprecision and Deming regression allows for the presence of error in both methods.<sup>577</sup>

When a regression analysis is performed, 95% confidence intervals of the slope and y-intercept should include 1 and 0, respectively to be considered acceptable.<sup>552</sup> If the y-intercept differs significantly from 0, constant bias is inferred. If the slope differs significantly from 1.0, proportional bias is inferred.<sup>578</sup> If there is a difference, this can be expressed as a percent e.g. 0.7 indicates 30% proportional error.<sup>578</sup>

In a Bland-Altman plot, the difference between the reference and test method is plotted on the y-axis, and the mean is plotted on the x-axis.<sup>578</sup> The mean difference and limits of agreement ( $\pm 1.96$  SD from the mean difference) are calculated and shown parallel to the x-axis.<sup>578</sup> If the methods are in agreement, the values should be distributed evenly around the zero difference line.<sup>578</sup> In addition,  $\geq 95\%$  of measured differences should fall within  $\pm 1.96$  SD from the mean difference.<sup>552,578</sup> Importantly, in both of these statistical techniques, clinical relevance and precision are not evaluated.<sup>552</sup>

After the method validation study is complete and the method deemed acceptable, species specific reference intervals should be established.<sup>552</sup>

## **METHOD VALIDATION OF LACTATE ANALYZERS IN DOGS**

Despite the widespread incorporation of lactate into veterinary emergency and critical care practice, a review of the literature revealed no evidence of method validation

in even the most commonly used benchtop lactate analyzers. Table 6 outlines the previously published literature validating lactate analyzers in dogs. As shown, the evidence to date has been composed primarily of precision and method comparison studies evaluating different field methods to benchtop methods used arbitrarily as reference methods. No published validation studies for the use of any of these benchtop methods in dogs were identified.

| Test Method(s)                                     | Nominated Reference Method        | Tests Performed                                                                            | Reference                       |
|----------------------------------------------------|-----------------------------------|--------------------------------------------------------------------------------------------|---------------------------------|
| Accutrend                                          | Rapidlab 865                      | Inter-assay precision<br>Intra-assay precision<br>Method comparison<br>Reference intervals | Stevenson 2007 <sup>511</sup>   |
| Lactate Scout                                      | Konelab 30i                       | Stability<br>Intra-assay precision<br>Method comparison                                    | Ferasin 2007 <sup>522</sup>     |
| Lactate Pro<br>Lactate Scout<br>Accutrend<br>iSTAT | Stat Profile Critical Care Xpress | Method comparison                                                                          | Acierno 2007 <sup>551</sup>     |
| Lactate Pro                                        | Nova StatProfile M                | Intra-assay precision<br>Inter-assay precision<br>Method comparison<br>Reference intervals | Thorneloe 2007 <sup>558</sup>   |
| Lactate Pro                                        | Roche/Hitachi 917                 | Method comparison<br>Reference intervals                                                   | Tas 2008 <sup>559</sup>         |
| iSTAT                                              | YSI 2300                          | Intra-assay precision<br>Method comparison                                                 | Berkman 2016 <sup>512</sup>     |
| Accutrend<br>iSTAT                                 | YSI 1500                          | Intra-assay precision<br>Method comparison                                                 | Karagiannis 2013 <sup>513</sup> |
| Lactate Plus                                       | GEM Premier 3000                  | Intra-assay precision<br>Method comparison                                                 | Nye 2017 <sup>554</sup>         |
| EPOC                                               | iSTAT                             | Intra-day precision                                                                        | West 2014 <sup>582</sup>        |

Table 6: Summary of previously published method validation studies for the measurement of lactate in dogs

The most plausible explanation is the lack of a well-defined “gold standard” method.<sup>513,521,554,558,561,583</sup> Despite this obstacle, an attempt at rigorous validation is prudent to strengthen the scientific foundation supporting lactate research in dogs. When a gold standard method is unknown or inaccessible, the next best representation of a “true” analyte quantity is a certified reference standard.<sup>552,558</sup> Certified reference materials satisfy international standards with known uncertainties and can therefore be used to evaluate bias.<sup>575</sup> Consequently, we designed a method validation and comparison study between a high-end, point-of-care benchtop analyzer and a benchtop clinical laboratory analyzer using canine plasma spiked with known quantities of certified reference standard sodium lactate (Chapter 4).

## CONCLUSION

Method validation is necessary before using a new analyzer in clinical practice. The first stage of method validation involves a series of tests to prove the method demonstrates adequate analytical performance. The majority of method validation studies are performed by comparing a new method to a definitive method (“gold-standard”), representing analytical “truth.” When a definitive method is unknown or unavailable, an alternative approach is to use known quantities of certified reference standard material to represent the “true values.” A review of the veterinary literature revealed that although lactate is commonly used in clinical practice, there have been no method validation studies published for the most commonly used and reported benchtop analyzers in dogs. As such, we designed a method validation study using recovery of certified reference standard sodium lactate to evaluate the analytical performance and equivalency of two benchtop reference lactate analyzers, presented in Chapter 4.

## CHAPTER 4: EVALUATION OF THE ANALYTICAL PERFORMANCE AND EQUIVALENCY OF A POINT-OF-CARE AND A CLINICAL LABORATORY ANALYZER FOR THE MEASUREMENT OF L-LACTATE IN CANINE PLASMA

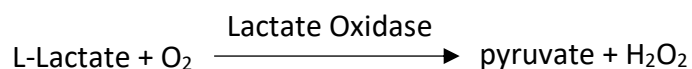
### INTRODUCTION

Veterinary practitioners have been measuring lactate concentrations in dogs in a clinical setting since the early 1990s. A variety of benchtop and handheld lactate analyzers have been developed for use in clinical practice during this time. Although numerous method comparison studies have evaluated novel handheld devices against benchtop analyzers as arbitrarily selected “gold standards,”<sup>511,551,552,558-560,573</sup> a rigorous evaluation of the analytical performance of these benchtop analyzers for use in dogs has not been published.<sup>558,561,573</sup> Analytical performance refers to the reliability (precision) and validity (accuracy, bias) of a particular analyzer in the measurement of a specified analyte.<sup>552</sup> As analytic performance can vary between species, species specific method validation is recommended.<sup>552</sup> The objectives of this study were to 1) assess the analytical performance of the Radiometer ABL 800 Basic (ABL800) in measuring lactate in canine plasma, 2) similarly assess the analytical performance of the Cobas Integra 400 Plus (Cobas400) and 3) evaluate equivalency between the two analyzers with a method comparison study. Analytical performance was assessed via investigating the linearity, precision, accuracy, total error and analytical range of each analyzer. We hypothesized that the Cobas400 would demonstrate superior analytical performance but that the two analyzers would be essentially equivalent for use in practice.

### MATERIALS AND METHODS

#### *Analyzers*

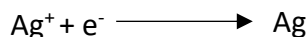
Two commercially available benchtop analyzers were investigated. The ABL800 measures lactate concentrations via enzyme amperometry. An electrode with a silver cathode and platinum anode is adjacent to a three-layer semi-permeable membrane. The analyzer draws 200 microliters of anticoagulated whole blood to contact the membrane. Lactate from the plasma fraction of the sample diffuses across the selectively permeable first layer of the membrane to contact the middle layer that is coated with the enzyme lactate oxidase. Lactate oxidase converts lactate into pyruvate and hydrogen peroxide via the following reaction:



The hydrogen peroxide diffuses across the inner layer of the membrane and contacts the platinum anode, where a second reaction occurs:

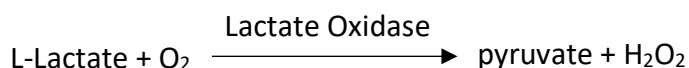


A voltage of 675 mV is applied to the electrode resulting in oxidation of the hydrogen peroxide and a measurable electrical current, which is proportionate to the amount of lactate in the sample. A silver cathode completes the electrical circuit by consuming electrons:<sup>584</sup>



The model used in this study processes one sample at a time, reports results in approximately 2 minutes and can process 18-24 samples per hour.<sup>585</sup> The manufacturer claims that the ABL800 can measure between 0.0-30 mmol/L and reports with acceptable analytical performance between 0.5-15 mmol/L.<sup>586</sup> Although the ABL800 uses whole blood as the sample medium, only the plasma fraction diffuses through the analyzer membrane and as such it actually measures and reports plasma lactate. The ABL800 reports to 1 decimal place at low and mid lactate concentrations but reports to 0 decimal places at high concentrations.

In contrast, the Cobas400 analyzer uses an enzymatic colorimetric method. It first draws up 2 microlitres of heparinized plasma. Next, lactate is oxidized by microbial lactate oxidase in the following reaction:



Peroxidase then catalyzes the reaction of hydrogen peroxide with ascorbate oxidase and 4-aminoantipyrine to produce a chromogen and water:



The intensity of colour determined via absorbance spectrophotometry at 552 nm is proportional to the amount of lactate in the sample.<sup>587</sup> The Cobas400 can process up to 400 tests per hour and is therefore typically used by clinical pathology laboratories. According to manufacturer claims, the Cobas400 can measure between 0.2-15.5 mmol/L with acceptable analytical performance but can offer a calculated extended measuring range of 0.2 and 155 mmol/L following sample dilution.<sup>587</sup> The recommended sample type for measurement of lactate on the Cobas400 is plasma separated within 15 minutes of collection.<sup>587</sup> The Cobas400 reports all results to 2 decimal places.

### ***Analyzer Maintenance***

Calibration and maintenance were performed as per manufacturers specifications for both analyzers.

For the ABL800, automated 2-point calibrations were performed once every 8 hours, and 1-point calibrations were performed every 4 hours. An additional 1-point calibration was performed whenever 11 measurements were made sequentially with an interval of less than ten minutes between samples. In addition, scheduled, automated cleaning occurred once every 8 hours. Decontamination was performed monthly, and protein removal was performed weekly. The lactate membrane was changed monthly, including 12 days prior to spiked sample analysis.<sup>584</sup>

For the Cobas400, quality control solutions were processed daily, probes cleaned weekly, and filters cleaned monthly. Calibration was performed whenever a new lactate reagent lot was used. Quality control solutions were processed after calibration and also once every 24 hours before commencement of a test run.

### ***Ethics***

Dogs used for blood sampling in this study were pets owned by staff of the University of Melbourne's Veterinary Teaching Hospital. All owners gave informed consent prior to enrollment. Animal handling and sampling was performed with full disclosure to and approval from the University of Melbourne's Animal Ethics Committee (approval number: 1413415; approved January 6, 2015).

### ***Test Matrix and Sample Processing***

#### ***Manufacturer Control Solutions***

Four concentrations (1.6, 4.5, 10.5 and 27 mmol/L) of quality control solutions (QualiCheck Control and High Metabolite Ampules; Radiometer Medical, Copenhagen, Denmark) were used in unaltered form. Radiometer manufactured control solutions were used on both analyzers because Cobas manufactured controls were only available at two concentration levels. For intra-assay precision and linearity, one operator per analyzer performed all replicates over 7 hours for the ABL800 and 1 hour for the Cobas400. For inter-assay precision, three operators processed samples on the Cobas400 and two operators processed samples on the ABL800 on 20 weekdays within a 30 day period.

#### ***Pooled Plasma***

During method validation, analyte solutions with matrices approximating real samples are preferred to saline dilution.<sup>578</sup> Certified reference standards for the measurement of blood or plasma lactate in dogs do not exist. Consequently, we created standard solutions using serial dilutions of 100% reference standard sodium lactate (USP: Rockville, MD; Lot # L0L443) with canine plasma. This ensured there were a sufficient number of samples across the entire reportable range.<sup>578</sup>

Because samples from individual animals can potentially cause unique matrix effects that could influence results,<sup>552</sup> plasma was sourced from 5 adult, healthy volunteer dogs and pooled for subsequent spiking and analysis (Table 7). Fresh frozen canine plasma was considered unsuitable because citrate, used as a routine anticoagulant during blood donation, has been shown to interfere with lactate measurement.<sup>523,524</sup> Consequently, whole blood was collected from each dog via jugular venipuncture. After obtaining a history and signed owner consent, each dog was examined and weighed. A 0.1 ml sample of whole blood was taken via direct venipuncture from the saphenous vein. The sample was placed into a microhematocrit tube and centrifuged to evaluate packed cell volume (PCV), total solids (TS) and plasma colour prior to donation. All 5 dogs were deemed eligible for inclusion as they were historically healthy, normal on physical examination and had PCV  $\geq 38\%$ , TS  $\geq 60$  g/L and visually clear plasma.

The hair surrounding and including the anticipated jugular puncture site was clipped to skin level and a small amount of 5% EMLA cream (25 mg/g Lignocaine, 25 mg/g Prilocaine) was applied at least 60 minutes prior to blood collection. Following aseptic skin preparation (clipping of fur followed by cleansing with 2% chlorhexidine solution and 70% isopropyl alcohol), a 22-gauge peripheral catheter was placed in the cephalic vein of all dogs other than dog 1, at the owner's request. All dogs also received 0.25 mg/kg butorphanol via the intravenous catheter prior to blood collection apart from dog 1, also at the owner's request. A 60 ml syringe and < 10 ml extension set were primed with 1015 USP units of lithium heparin (Sigma-Aldrich, Castle-Hill, NSW; lot# SLBK2401V, 203 USP units/mg) diluted in 10 ml of 0.9% saline. The acceptable range of heparin in lithium heparin collection tubes is 10-30 USP units/ml of blood.<sup>588-590</sup> Consequently, after aseptic skin preparation, an 18-gauge needle was attached to the extension set, and the assembly was used to withdraw 35 to 50 ml of blood per 60 cc syringe to achieve appropriate heparin concentrations. Blood sampling was performed without complications, however only 45 ml was collected from dog 1 due to unexplained cessation of flow. The blood from each individual syringe was then injected into one 600 ml commercial dry blood collection bag per dog. (Jorgensen Labs, Loveland Colorado) Individual donations resulted in calculated lithium heparin concentrations ranging from 20 and 27 USP units per ml of blood (Table 7). The blood was then separated using a veterinary blood-bank purposed large-capacity, temperature-controlled centrifuge (4°C, 5000 g, 7 minutes) and plasma extractor. The plasma was transferred into a new dry blood collection bag and sealed with a heat sealer and aluminum sealing clips. The cellular fraction was discarded and the plasma was stored in a -80°C freezer. The dogs were monitored uneventfully for 4 hours following blood collection and then discharged to their respective owners. Plasma from the 5 dogs was stored for one to eight days and then thawed and manually combined into a new dry blood collection bag using a 60 cc syringe and 18-gauge needle, resulting in 390 ml of pooled plasma. The bag was sealed with a heat sealer and aluminum sealing clips and refrozen at -80°C until further use.

| # | Date (D/M/Y) | Age | Breed    | Sex | Weight (kg) | Physical Exam | Collected Whole Blood (ml) | Total LH (USP Units) | [LH] (USP Units/ml blood) | Plasma Volume (ml) |
|---|--------------|-----|----------|-----|-------------|---------------|----------------------------|----------------------|---------------------------|--------------------|
| 1 | 21/08/15     | 2   | Lab X    | FS  | 19.4        | Normal        | 45                         | 1015                 | 22                        | 20                 |
| 2 | 22/08/15     | 6   | Kelpie X | MN  | 20.3        | Normal        | 160                        | 4060                 | 25                        | 35                 |
| 3 | 22/08/15     | 3   | Boxer X  | MN  | 26.0        | Normal        | 190                        | 5075                 | 27                        | 45                 |
| 4 | 27/08/15     | 3   | Lab      | MN  | 33.3        | Normal        | 250                        | 5075                 | 20                        | 140                |
| 5 | 27/08/15     | 6   | G. Ret   | MN  | 32.4        | Normal        | 250                        | 5075                 | 20                        | 150                |

FS – Female Spayed, MN – Male Neutered, X – Crossbreed, LH – Lithium Heparin

Table 7: Summary of blood collection and anticoagulation to create pooled plasma

The source of lactate for this study was USP reference standard 100% sodium lactate soluble powder without specified impurities.(USP: Rockville, MD; Lot # LOL443) This product is considered stable under normal conditions but can be degraded if mixed with a strong oxidizing substance.<sup>591</sup> The sodium lactate was stored in a tightly closed container, protected from light and refrigerated until use as per USP specifications.<sup>592</sup> Using a precision gram scale, four series of 50% stepwise dilutions starting at 40, 32, 28 and 24 mmol/L were performed to create an appropriate range of concentrations for testing (Baseline + 0.25-40 mmol/L; 23 concentrations in total).(Appendix C) Spiking and stepwise dilutions were completed within one 8 hour period. Samples were stored overnight in closed containers at 4°C. Experimental analysis began the following morning and were completed within 20 hours. Previous stability testing has demonstrated that lactate is stable in separated human plasma anticoagulated with lithium heparin for 56 hours at room temperature.<sup>593</sup> In addition, the Cobas lactate test package insert advises that lactate is stable in separated plasma for 8 hours at room temperature (15 – 25 °C) or 14 days if refrigerated (2 – 8 °C).<sup>587</sup>

During processing, samples were kept capped and refrigerated at 4°C in between use. A formal sample randomization protocol was not used as part of this study. For the ABL800, each plasma sample was processed individually by a single operator. An attempt was made to vary sample order with no more than 2 sequential replicates per concentration level. During the last 3 hours of sample analysis, however, the final 4 of 20 replicates were performed sequentially at 8 concentration levels due to time constraints. Samples were processed continuously on the ABL800 for 20 hours. For the Cobas400, plasma samples were processed in batches of up to 120 tests a time by a single operator. All replicates per concentration level were performed in the same batch. While performed on the same day as testing on the ABL800, sample processing was completed within 3 hours.

### ***Evaluation of Analytical Performance***

All validation procedures satisfied the minimum recommended methods for

validation described by the American Society for Veterinary Clinical Pathology (ASVCP).<sup>552,578</sup> Other consulted resources included: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) documents Q2(R1): Validation of Analytical Procedures,<sup>562</sup> the International Union of Pure and Applied Chemistry Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis<sup>575</sup> and the Bioanalytical Method Validation Guidance for Industry.<sup>576</sup>

### Manufacturer Controls

Linearity and precision were first evaluated using manufacturer control solutions to ensure the analyzers were performing adequately. Linearity was evaluated on both analyzers using three levels of control solutions manufactured by Radiometer (1.6, 4.5, and 10.5 mmol/L). To evaluate intra-assay precision (repeatability), 20 replicates were performed in one day on four levels of Radiometer control solutions (1.6, 4.5, 10.5 and 27 mmol/L). Inter-assay precision (reproducibility) was then evaluated by performing 20 replicates over 20 days using four levels of Radiometer control solutions (1.6, 4.5, 10.5 and 27 mmol/L).

### Spiked Canine Plasma

#### *Baseline*

Canine plasma inherently contains a small amount of endogenous lactate. Twenty replicates of pooled canine plasma without the addition of sodium lactate were processed on each analyzer to establish analyzer-specific mean baseline lactate concentrations (X).

#### *Linearity*

Linearity was evaluated using manually spiked canine plasma at 17 concentrations encompassing 0 to >150% of the range encountered in clinical practice and exceeding the manufacturer reported analytical ranges.<sup>575</sup> The minimum of 3 replicates (IUPAC 2002) were performed at 5 concentrations (X+6.0, X+8.0, X+12.0, X+32.0, X+40) and the mean of each was used for analysis. As 20 replicates at the remaining concentrations (X+0.25, X+0.5, X+1.0, X+1.5, X+2.0, X+2.5, X+3.0, X+3.5, X+4.0, X+5.0, X+7.0, X+10.0, X+16, X+20) were performed for precision analysis, the mean of the 20 replicates was used in linearity assessment. Linearity was plotted as mean measured lactate concentration vs. expected lactate concentration (analyzer specific baseline [lactate] + spiked [lactate]). Acceptance was based on visual inspection<sup>578</sup> as well as the exclusion of outliers and curvature of residuals.<sup>575</sup>

#### *Precision*

Intra-assay precision was evaluated by measuring 20 replicates at 16 concentrations of spiked plasma (X, X+0.25, X+0.5, X+1.0, X+1.5, X+2.0, X+2.5, X+3.0, X+3.5, X+4.0, X+5.0,

X+7.0, X+10.0, X+16.0, X+20.0, X+32.0). One operator per analyzer performed all measurements within the same 24-hour period. Total measured lactate concentrations (analyzer specific baseline [lactate] + spiked [lactate]) were used for analysis. Coefficients of variation (CVs) were considered acceptable if < 13.6%.<sup>580,582</sup>

### *Accuracy (Bias)*

As a gold standard analyzer has not been established for the measurement of lactate in canine plasma, bias was evaluated by performing spike and recovery analysis.<sup>577</sup> The percent recovery was calculated at 19 different concentrations, with replicates and concentrations as described for linearity as well as 20 replicates at X+16.0, 20 replicates at X+32.0 and 3 replicates at X+40.0. Baseline lactate was subtracted from the measured concentration at each data point, such that only the known, spiked concentrations were used in analysis. Performance was considered acceptable if % systematic error (SE) was less than 8%.<sup>580</sup>

Calculations were performed as follows:

$$(1a) \text{ Percent recovery} = ([\text{mean}_{\text{measured lactate}}] - [\text{mean}_{\text{baseline lactate}}]) / [\text{spiked lactate}] \times 100\%$$

$$(1b) \text{ Systematic error} = 100\% - |\text{percent recovery}|$$

### *Total Error*

The following calculation was used to determine the total error of each analyzer using spiked canine plasma at 15 concentrations (X+0.25, X+0.5, X+1.0, X+1.5, X+2.0, X+2.5, X+3.0, X+3.5, X+4.0, X+5.0, X+7.0, X+10.0, X+16.0, X+20.0, X+32.0):

$$(2) \text{ TE}_{\text{obs}} = \% \text{ Bias} + 2\text{CV}$$

CV was obtained from precision analysis, based on total measured concentrations (analyzer specific baseline [lactate] + spiked [lactate]). Percent bias was obtained from accuracy analysis, based on spiked concentrations excluding baseline lactate to allow comparison between analyzers. Total error was considered acceptable if the observed total error (TE<sub>obs</sub>) was less than ASVCP pre-defined acceptable total error (TE<sub>a</sub>) for lactate of 40%.<sup>579,580</sup>

### *Reportable Range*

The reportable range was determined for both analyzers by determining the highest and lowest points within the linear range of the assay where CV was < 13.6% and the %SE < 8%.

### *Comparison of Methods*

Method comparison was performed using the Cobas400 as the designated reference method. The methods were compared at 16 different concentrations, with replicates and concentrations as described for linearity, excluding X+16.0 due to measurement error. Given the baseline lactate concentration differed between analyzers, the baseline concentration was subtracted from each measured value such that the concentration of only the spiked component was compared (ranging from 0.25 to 20 mmol/L).

Results were evaluated using Passing-Bablok linear regression and Bland-Altman analysis. As per convention, on the Bland-Altman plot, the difference in measurements from the two methods was plotted on the Y-axis, and the mean was plotted on the X-axis. The methods were considered in agreement if the values were distributed evenly around the zero difference line and if  $\geq 95\%$  of measured differences fell within  $\pm 1.96$  SD of the mean difference.<sup>552,578</sup>

### ***Statistical Analysis***

All analyses were conducted using Stata V14 (StataCorp, State College, TX). Correlations, Ordinary Least Squares as well as mean, standard deviations, and coefficients of variation were obtained using standard Stata modules. Method comparison was carried out using 'agree', a Stata package specifically written by JM Doménech in Stata for comparing a reference to a standard method. "Agree" computes Passing-Bablok and Bland-Altman analyses and provides standard statistics and charts to compare two methods.<sup>594</sup>

## **RESULTS**

### **Manufacturer Control Solutions**

Linearity for both analyzers was determined to be adequate. The ABL800 had a slope of 1.01 (95% CI: 0.84, 1.19) and a Y-intercept of 0.07 (95% CI: -1.12, 1.26) ( $r > 0.999$ ,  $r^2 > 0.999$ ) (Figure 7, Appendix 4). The Cobas400 had a slope of 1.14 (95% CI: 0.87, 1.41) and a Y-intercept of 0.17 (95% CI: -1.64, 1.98) ( $r > 0.999$ ,  $r^2 = 0.999$ ) (Figure 7, Appendix 4).

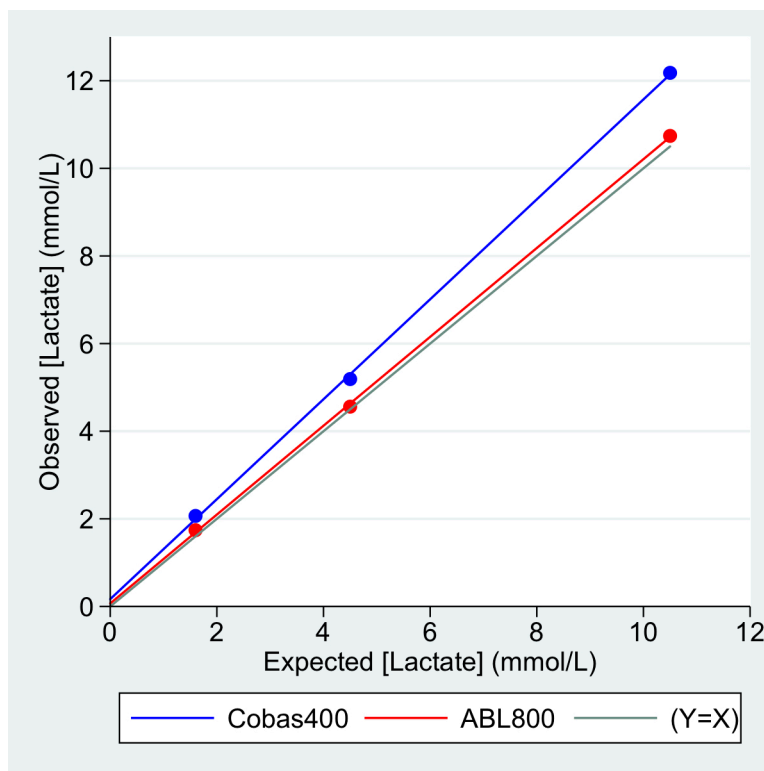


Figure 7: Linearity using Radiometer manufactured control solutions

Precision for both analyzers was also adequate. The ABL800 produced intra-assay CVs ranging from 1.8 to 2.9%, and inter-assay CVs ranging from 2.2 to 5.3% (Table 8). The intra-assay CVs for the Cobas400 using the Radiometer control solutions ranged from 0.4 to 1.3% while the inter-assay CVs ranged from 1.8 to 4.0% (Table 9).

| Expected<br>[Lactate]<br>(mmol/L) | Radiometer ABL 800            |                                   |           |                               |                                   |           |
|-----------------------------------|-------------------------------|-----------------------------------|-----------|-------------------------------|-----------------------------------|-----------|
|                                   | Intra-assay                   |                                   |           | Inter-assay                   |                                   |           |
|                                   | Mean<br>[lactate]<br>(mmol/L) | Standard<br>Deviation<br>(mmol/L) | CV<br>(%) | Mean<br>[lactate]<br>(mmol/L) | Standard<br>Deviation<br>(mmol/L) | CV<br>(%) |
| 1.6                               | 1.74                          | 0.05                              | 2.9       | 1.66                          | 0.07                              | 4.1       |
| 4.5                               | 4.56                          | 0.12                              | 2.6       | 4.54                          | 0.15                              | 3.2       |
| 10.5                              | 10.74                         | 0.25                              | 2.3       | 10.41                         | 0.23                              | 2.2       |
| 27                                | 28.50                         | 0.51                              | 1.8       | 29.35                         | 1.56                              | 5.3       |

Table 8 – Intra- and Inter-assay precision using Radiometer control solutions for ABL 800

| Expected<br>[Lactate]<br>(mmol/L) | Cobas Integra 400             |                                   |           |                               |                                   |           |
|-----------------------------------|-------------------------------|-----------------------------------|-----------|-------------------------------|-----------------------------------|-----------|
|                                   | Intra-assay                   |                                   |           | Inter-assay                   |                                   |           |
|                                   | Mean<br>[lactate]<br>(mmol/L) | Standard<br>Deviation<br>(mmol/L) | CV<br>(%) | Mean<br>[lactate]<br>(mmol/L) | Standard<br>Deviation<br>(mmol/L) | CV<br>(%) |
| 1.6                               | 2.07                          | 0.01                              | 0.5       | 2.08                          | 0.04                              | 2.0       |
| 4.5                               | 5.19                          | 0.02                              | 0.4       | 5.24                          | 0.10                              | 1.9       |
| 10.5                              | 12.18                         | 0.07                              | 0.6       | 12.28                         | 0.22                              | 1.8       |
| 27                                | 24.06                         | 0.32                              | 1.3       | 23.81                         | 0.97                              | 4.0       |

Table 9 – Intra- and Inter-assay precision using Radiometer control solutions for Cobas400

### Spiked Canine Plasma

#### *Baseline*

The mean lactate concentration in the pooled canine plasma prior to the addition of sodium lactate was  $1.82 \pm 0.04$  (mean $\pm$ SD) mmol/L for the ABL800 and  $1.91 \pm 0.01$  (mean $\pm$ SD) mmol/L for the Cobas400.

#### *Linearity*

Based on visual assessment, data point X+32.0 was deemed an outlier and was excluded from further analysis. This was not unexpected given this data point is well above the analytical range reported by the manufacturers. In addition, 16 replicates were included in statistical analysis for one ABL800 concentration (X+4.0) and one Cobas400 concentration (X+20) due to measurement errors as specified in Table 10. Two concentrations, X+16 and X+40, were excluded from analysis due to consistently erroneous measurements on the Cobas400 and ABL800, respectively (Appendix 14, 15). Linear regression for the ABL800 revealed a slope of 0.87 (95% CI: 0.85, 0.89) and a Y-intercept of 0.50 (95% CI: 0.35, 0.66) ( $r > 0.999$ ,  $r^2 = 0.998$ ) over the range of 2.07 to 21.82 (X+0.25 to X+20.0) mmol/L (Figure 8). For the Cobas400 the slope was 0.97 (95% CI: 0.95, 0.99) and the Y-intercept was 0.31 (95% CI: 0.11, 0.50) ( $r > 0.999$ ,  $r^2 = 0.998$ ) over the range of 2.16 to 21.91 (X+0.25 to X+20.0) mmol/L (Figure 8). In this range, linearity was acceptable for both analyzers. Minor curvature of residuals was observed, however, it was trivial enough that transformation of data was not required.

Linearity was better within ranges of greater clinical relevance. For the ABL800, between 2.07 and 6.82 (x+0.25 to X+5.0) mmol/L, slope and intercept were 0.97 (95% CI: 0.95, 0.99) and 0.12 (95% CI: 0.05, 0.18), respectively ( $r > 0.999$ ,  $r^2 > 0.999$ ). For the Cobas400, between 2.16 and 7.91 (X+0.25 to X+6.0) mmol/L, slope and intercept were 1.04 (95% CI: 1.03, 1.05) and -0.03 (95% CI: -0.08, 0.02), respectively ( $r > 0.9999$ ,  $r^2 > 0.999$ ). In

addition, within these ranges there was no evidence of curvature of the line of residuals.

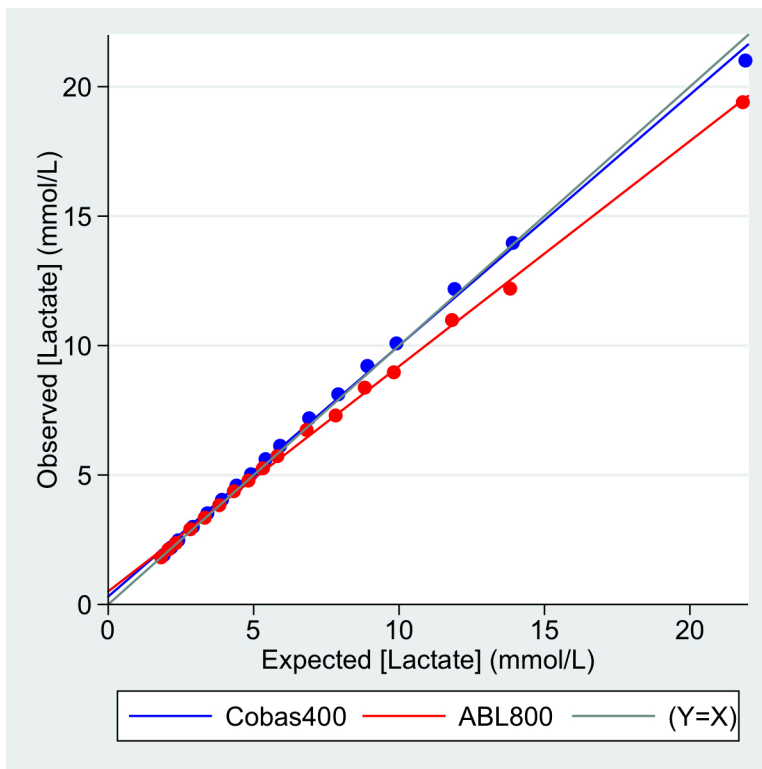


Figure 8: Linearity of Cobas400 and ABL800 for lactate measurement on spiked canine plasma

## Precision

Precision was excellent at all concentrations for both analyzers. Between lactate concentrations 1.8 (X+0) and 21.82 (X+20.0) mmol/L, intra-assay CVs on the ABL800 ranged from 0.0 to 3.1%. Between lactate concentrations 1.91 (X+0) and 21.91 (X+20.0) mmol/L, intra-assay CVs on the Cobas400 ranged from 0.3 to 2.2% (Table 10).

| Spiked [lactate] (mmol/L) | ABL800 |                             |                                  |             |        | Cobas400 |                             |                                  |             |        |
|---------------------------|--------|-----------------------------|----------------------------------|-------------|--------|----------|-----------------------------|----------------------------------|-------------|--------|
|                           | n      | Expected [lactate] (mmol/L) | Mean Measured [lactate] (mmol/L) | SD (mmol/L) | CV (%) | n        | Expected [Lactate] (mmol/L) | Mean Measured [lactate] (mmol/L) | SD (mmol/L) | CV (%) |
| Baseline (X)              | 20     | N/A                         | 1.82                             | 0.04        | 2.2    | 20       | N/A                         | 1.91                             | 0.01        | 0.4    |
| X + 0.25                  | 20     | 2.07                        | 2.12                             | 0.06        | 3.0    | 20       | 2.16                        | 2.19                             | 0.01        | 0.4    |
| X + 0.5                   | 20     | 2.32                        | 2.36                             | 0.07        | 2.8    | 20       | 2.41                        | 2.48                             | 0.01        | 0.4    |
| X + 1.0                   | 20     | 2.82                        | 2.90                             | 0.05        | 1.8    | 20       | 3.91                        | 3.00                             | 0.01        | 0.4    |
| X + 1.5                   | 20     | 3.32                        | 3.35                             | 0.06        | 1.8    | 20       | 3.41                        | 3.52                             | 0.01        | 0.4    |
| X + 2.0                   | 20     | 3.82                        | 3.84                             | 0.07        | 1.9    | 20       | 3.91                        | 4.04                             | 0.02        | 0.4    |
| X + 2.5                   | 20     | 4.32                        | 4.38                             | 0.08        | 1.9    | 20       | 4.41                        | 4.59                             | 0.02        | 0.4    |
| X + 3.0                   | 20     | 4.82                        | 4.78                             | 0.07        | 1.4    | 20       | 4.91                        | 5.03                             | 0.02        | 0.3    |
| X + 3.5                   | 20     | 5.32                        | 5.26                             | 0.09        | 1.8    | 20       | 5.41                        | 5.61                             | 0.02        | 0.4    |
| X + 4.0                   | 16*    | 5.82                        | 5.73                             | 0.14        | 2.4    | 20       | 5.91                        | 6.13                             | 0.02        | 0.3    |
| X + 5.0                   | 20     | 6.82                        | 6.74                             | 0.16        | 2.4    | 20       | 6.91                        | 7.19                             | 0.03        | 0.4    |
| X + 7.0                   | 20     | 8.82                        | 8.38                             | 0.16        | 1.9    | 20       | 8.91                        | 9.21                             | 0.05        | 0.6    |
| X + 10.0                  | 20     | 11.82                       | 10.98                            | 0.26        | 2.3    | 20       | 11.91                       | 12.18                            | 0.04        | 0.3    |
| X + 16.0                  | 20     | 17.82                       | 16.00                            | 0.00        | 0.0    | 20       | 17.91                       | Error**                          | N/A         | N/A    |
| X + 20.0                  | 20     | 21.82                       | 19.40                            | 0.60        | 3.1    | 16*      | 21.91                       | 21.01                            | 0.15        | 0.7    |
| X + 32.0                  | 20     | 33.82                       | 28.90                            | 0.64        | 2.2    | 20       | 33.91                       | 26.55                            | 0.60        | 2.2    |

\*\*All 20 replicates reported as ">15.50 mmol/L"

\*16 replicates included in analysis (4 replicates reported as ">15.50 mmol/L" by the Cobas400, "sample error" reported for 4 replicates on the ABL800)

Table 10 – Inter-assay precision using spiked canine plasma

### Accuracy (Bias)

The ABL800 satisfied the criteria for acceptance ( $SE \leq 8\%$ ) between lactate concentrations  $X+0.5$  and  $X+5.0$  mmol/L. The Cobas400 satisfied the criteria for acceptance between lactate concentrations  $X+1.5$  and  $X+20$  mmol/L (Table 11).

| Expected recovery (mmol/L) | ABL800 |                             |              |                      | Cobas400 |                             |              |                      |
|----------------------------|--------|-----------------------------|--------------|----------------------|----------|-----------------------------|--------------|----------------------|
|                            | n      | Mean Measured [lactate] – X | Recovery (%) | Systematic Error (%) | n        | Mean Measured [lactate] – X | Recovery (%) | Systematic Error (%) |
| 0.25                       | 20     | 0.30                        | 120.0        | 20.0                 | 20       | 0.28                        | 113.8        | 13.8                 |
| 0.5                        | 20     | 0.54                        | 108.0        | 8.0                  | 20       | 0.57                        | 113.5        | 13.5                 |
| 1.0                        | 20     | 1.08                        | 108.0        | 8.0                  | 20       | 1.09                        | 108.7        | 8.7                  |
| 1.5                        | 20     | 1.53                        | 102.0        | 2.0                  | 20       | 1.61                        | 107.4        | 7.4                  |
| 2.0                        | 20     | 2.02                        | 100.8        | 0.8                  | 20       | 2.13                        | 106.6        | 6.6                  |
| 2.5                        | 20     | 2.56                        | 102.2        | 2.2                  | 20       | 2.68                        | 107.2        | 7.2                  |
| 3.0                        | 20     | 2.97                        | 98.8         | 1.2                  | 20       | 3.12                        | 104.0        | 4.0                  |
| 3.5                        | 20     | 3.44                        | 98.3         | 1.7                  | 20       | 3.70                        | 105.8        | 5.8                  |
| 4.0                        | 16*    | 3.91                        | 97.8         | 2.2                  | 20       | 4.22                        | 105.5        | 5.5                  |
| 5.0                        | 20     | 4.92                        | 98.4         | 1.6                  | 20       | 5.28                        | 105.6        | 5.6                  |
| 6.0                        | 3      | 5.48                        | 91.3         | 8.7                  | 3        | 6.21                        | 103.4        | 3.4                  |
| 7.0                        | 20     | 6.56                        | 93.6         | 6.4                  | 20       | 6.55                        | 93.6         | 6.4                  |
| 8.0                        | 3      | 7.15                        | 89.3         | 10.7                 | 3        | 8.17                        | 102.2        | 2.2                  |
| 10.0                       | 20     | 9.16                        | 91.6         | 8.4                  | 20       | 10.27                       | 102.7        | 2.7                  |
| 12.0                       | 3      | 10.38                       | 86.5         | 13.5                 | 3        | 12.06                       | 100.5        | 0.5                  |
| 16.0                       | 20     | 14.18                       | 88.6         | 11.4                 | 20       | Error**                     | N/A          | N/A                  |
| 20.0                       | 20     | 17.58                       | 87.9         | 12.1                 | 16*      | 19.10                       | 95.5         | 4.5                  |
| 32.0                       | 20     | 27.08                       | 84.6         | 15.4                 | 20       | 24.64                       | 77.0         | 23.0                 |
| 40.0                       | 3      | Error***                    | N/A          | N/A                  | 3        | 26.96                       | 67.39        | 32.6                 |

\*\*\* All 3 replicates reported as “High” by the ABL800

\*\*All 20 replicates reported as “>15.50 mmol/L” by the Cobas400

\*16 replicates included in analysis (4 replicates reported as “>15.50 mmol/L” by the Cobas400, “sample error” reported for 4 replicates on the ABL800)

Table 11 – Accuracy via spike and recovery analysis using canine plasma

### Total Error

As, at all tested concentrations, both the Cobas400 and the ABL800 satisfied the criteria for acceptance specified by the ASVCP ( $TE_{obs} < 40\%$ ), analytical performance was considered acceptable for both analyzers (Table 12).<sup>578,579</sup>

| Expected [Lactate] (mmol/L) | ABL800 |        |        |            | Cobas400 |        |        |            |
|-----------------------------|--------|--------|--------|------------|----------|--------|--------|------------|
|                             | n      | CV (%) | % Bias | $TE_{obs}$ | n        | CV (%) | % Bias | $TE_{obs}$ |
| Baseline + 0.25             | 20     | 3.0    | 20.0   | 26.0       | 20       | 0.4    | 13.8   | 14.6       |
| Baseline + 0.5              | 20     | 2.8    | 8.0    | 13.7       | 20       | 0.4    | 13.5   | 14.4       |
| Baseline + 1.0              | 20     | 1.8    | 8.0    | 11.5       | 20       | 0.4    | 8.7    | 9.4        |
| Baseline + 1.5              | 20     | 1.8    | 2.0    | 5.6        | 20       | 0.4    | 7.4    | 8.2        |
| Baseline + 2.0              | 20     | 1.9    | 0.8    | 4.6        | 20       | 0.4    | 6.6    | 7.3        |
| Baseline + 2.5              | 20     | 1.9    | 2.2    | 6.1        | 20       | 0.4    | 7.2    | 8.2        |
| Baseline + 3.0              | 20     | 1.4    | 1.2    | 4.0        | 20       | 0.3    | 4.0    | 4.6        |
| Baseline + 3.5              | 20     | 1.8    | 1.7    | 5.3        | 20       | 0.4    | 5.8    | 6.5        |
| Baseline + 4.0              | 16     | 2.3    | 2.2    | 6.9        | 20       | 0.3    | 5.5    | 6.2        |
| Baseline + 5.0              | 20     | 2.4    | 1.6    | 6.4        | 20       | 0.4    | 5.6    | 6.4        |
| Baseline + 7.0              | 20     | 1.9    | 6.4    | 10.1       | 20       | 0.6    | 4.3    | 5.5        |
| Baseline + 10.0             | 20     | 2.3    | 8.4    | 13.0       | 20       | 0.3    | 2.7    | 3.4        |
| Baseline + 16.0             | 20     | 0      | 11.4   | 11.4       | 20       | Error  | N/A    | N/A        |
| Baseline + 20.0             | 20     | 3.1    | 12.1   | 18.3       | 16       | 0.7    | 4.5    | 5.9        |
| Baseline + 32.0             | 20     | 2.2    | 15.4   | 19.8       | 20       | 2.2    | 23.0   | 27.5       |

Table 12 – Analysis of analyzer total error

### Reportable Range

Based on acceptance criteria of CV less than 13.6% and percentage bias  $\leq 8\%$ , the reportable range for the ABL 800 is 2.3 to 6.8 (X+0.5 to X+5.0) mmol/L and for the Cobas is 3.41 to 21.91 mmol/L (X+1.5 to X+20.0 mmol/L).

### Comparison of Methods

Upon visual inspection, the 32.0 mmol/L data point was deemed an outlier and excluded in subsequent analysis. Passing-Bablok regression over the range 0.25 – 20 mmol/L (Figure 9) revealed a slope of 0.89 (95% CI 0.86, 0.92) and Y-intercept of 0.11 (95% CI: 0.04, 0.22), demonstrating the presence of 11% proportional bias and 0.11 mmol/L constant bias.

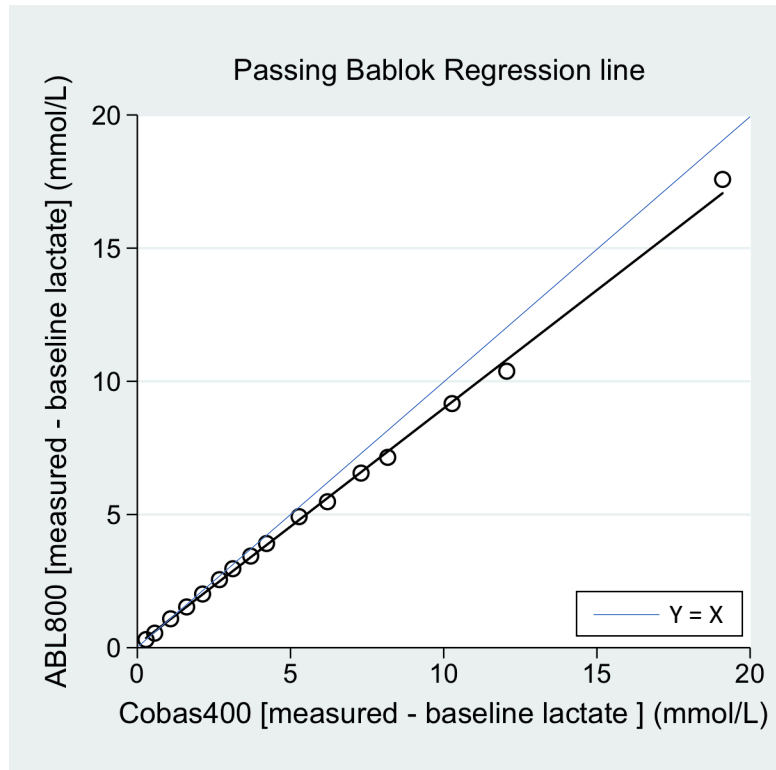


Figure 9: Passing-Bablok regression analysis of measured spiked lactate concentrations in canine plasma by the ABL800 (y-axis) and the Cobas400 (x-axis)

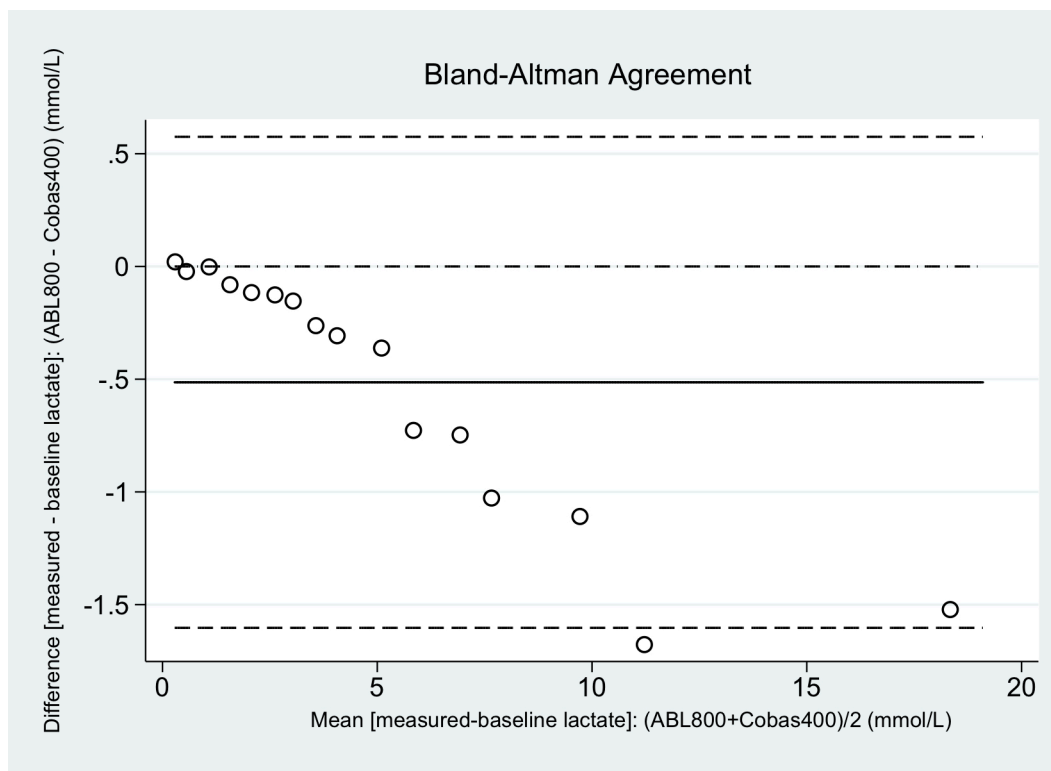


Figure 10: Bland-Altman plot with superimposed mean difference (solid line)  $\pm$  1.96SD (dashed lines)

On the Bland-Altman plot, the mean difference was -0.51 (95% CI: -1.6, 0.58). A small but clinically significant negative constant bias was observed for the ABL800 relative to the Cobas400. Proportional bias was also evident by the asymmetric distribution of data points around zero and the greater scatter of data points at higher concentrations.<sup>552,578</sup> The proportional bias was negative for the ABL800 relative to the Cobas400. As  $\approx$  95% (15/16) of differences fell within  $\pm$  1.96 SD of the mean difference, the ABL800 can be considered acceptable for clinical use when compared to the Cobas400.<sup>552</sup> However, given the presence of constant and proportional bias, the methods cannot be considered equivalent.

## DISCUSSION

This study established that while both analyzers demonstrated acceptable analytical performance for use in clinical practice, the Cobas400 performed slightly better than the ABL800.

Linearity and precision were first evaluated using manufacturer control solutions to ensure performance was sufficient for subsequent testing with spiked canine plasma.

Although the Cobas400 was more precise than the ABL800, both analyzers demonstrated excellent precision at all tested levels. In addition, both analyzers demonstrated adequate linearity as the 95% confidence intervals for slope and intercept included 1 and 0, respectively and  $r^2$  was greater than 0.999 for both analyzers. In contrast to other findings in this study, the ABL800 showed superior linearity to the Cobas400 with a slope and intercept that more closely approximated 1 and 0, respectively, as well as tighter 95% confidence intervals. This does not, however, indicate that the ABL800 is a superior analyzer because control solutions manufactured by Radiometer were used on both the ABL800 and the Cobas400. Consequently, the Cobas400 might have been at an analytical disadvantage for this measure of analytical performance. It would have been preferable to use Radiometer manufactured control solutions on the ABL800 and Cobas manufactured control solutions on the Cobas400, however, only two levels of Cobas manufactured control solutions were available when this study was performed, which would have been insufficient for linearity assessment.

Although both analyzers demonstrated excellent precision using spiked canine plasma, the Cobas400 was more precise than the ABL800 at all tested concentrations. It is possible that this finding is at least in part due to the fact that the Cobas400 reports to 2 decimal places whereas the ABL800 only reports to either 1 or 0 decimal places. Additionally, the fact that the ABL800 reports to 0 decimal points at higher concentrations may have influenced data at the X+16.0 mmol/L concentration, which was collected successfully on only the radiometer (Table 10). Precision at this concentration was deemed to be 100%, which is unlikely given 100% precision was not observed at any other concentration. One possible explanation is that the results actually fell within 15.6 mmol/L and 16.4 mmol/L such that rounding error resulted in an erroneous conclusion of 100% precision at this data point. This was not observed at lower concentrations where 1 decimal point was reported, or at higher concentrations where variation was large enough that rounding to the prior or next whole number occurred. Precision using spiked canine plasma was better on both analyzers than previously reported data for hand-held devices using samples collected from dogs, including the: Accutrend (CVs: 1.9, 4.7, 4.8, 6.4, 10.3%),<sup>513</sup> Lactate Plus (CV range: 0-26.2%, median 3.7%),<sup>554</sup> Lactate Pro (CVs: 1.7, 4.1, 5.2%)<sup>558</sup> and EPOC (CV: 6.8%).<sup>582</sup> The YSI 2300 was the only benchtop analyzer with published precision data using canine samples, for which CVs were reported as 1.27 and 1.77%, slightly superior to the ABL800 and slightly inferior to the Cobas400.<sup>512</sup>

Overall, the Cobas400 was also more accurate than the ABL800. The Cobas400 met acceptable bias limits ( $SE \leq 8\%$ ) between 3.41 and 21.91 (X+1.5 to X+20.0) mmol/L whereas the ABL800 only satisfied this requirement between 2.3 and 6.8 (X+0.5 to X+5.0) mmol/L. Interestingly, at every concentration tested between 2.3/2.41 and 6.8/6.91 (X+0.5 to X+5.0) mmol/L (ABL800/Cobas400), the ABL800 was more accurate. Clinical decision making points for lactate in dogs vary substantially in the veterinary literature (Tables 2 and 3). The majority, however, fall between 2.5 and 6.5 mmol/L. Accordingly, the ABL800 is more accurate than the Cobas400 within arguably the most important clinical range. However total error, discussed below, is of greater clinical relevance as both accuracy and precision

impact clinical test results. Although accuracy for the measurement of lactate via spike and recovery has not been previously published using canine plasma, a similar study was performed using bovine albumin-based commercial control solutions on a predecessor to the ABL800, the Radiometer EML105 (Radiometer Medical, Copenhagen, Denmark).<sup>595</sup> When compared to the Hitachi 911 (Boehringer Mannheim, Mannheim, Germany), bias became increasingly negative at high concentrations, with % recovery of approximately 90% at 12 mmol/L, 87% at 15.0 and 85% at 20.0 mmol/L. This was very similar to the results of our study.

Based on %CV and %SE limits of 13.6 and 8%, respectively,<sup>580</sup> we determined the reportable range for the ABL 800 to be 2.3 to 6.8 ( $X+0.5$  to  $X+5.0$ ) mmol/L and 3.41 to 21.91 mmol/L ( $X+1.5$  to  $X+20.0$  mmol/L) for the Cobas400. These acceptance limits were obtained from a reference designed for human clinical laboratory method validation based on biological variation.<sup>580</sup> Biological variation-based quality requirements are generally considered too stringent for veterinary diagnostic instruments, and as such the ASVCP uses clinical consensus to establish acceptance limits based on clinical decision limits rather than biological variation.<sup>579</sup> While the ASVCP  $TE_a$  limit for lactate of 40% is comparable to  $TE_a$  limits specified by other organizations,<sup>579,580</sup> the minimum ASVCP limits for precision and accuracy are more lenient ( $\%SE < TE_a$  for accuracy via recovery and  $\%CV < TE_a$  for precision)<sup>578</sup> By using the minimum ASVCP limits, the reportable range for both the ABL800 and the Cobas400 would have encompassed all tested concentrations (i.e.  $X+0.25$  to  $X+32.0$  for the ABL800 and  $X+0.25$  to  $X+40.0$  for the Cobas400), exceeding the manufacturers' analytical ranges. A %CV or % SE limit of 40% could, however, allow enough variability to compromise clinical interpretation (i.e. with a %CV or %SE of 40%, a patient with a true lactate concentration of 5 mmol/L could measure anywhere between 3 and 7 mmol/L). As the limits established by Ricos et al.<sup>580</sup> were more appropriate for clinical decision making, we elected to use 13.6 and 8% as our threshold for acceptance of precision and accuracy, respectively.

Both the Cobas400 and the ABL800 satisfied the ASVCP total error requirement of  $TE_{obs} < TE_a$  (40%) at all tested concentrations. Consequently, both analyzers have acceptable analytical performance for use in clinical practice (Table 12).<sup>552,558</sup> Furthermore, either method can now be used as a reference method for future validation studies. It is important to acknowledge that if a more stringent level is selected for  $TE_a$ , such as 10% as recommended by the CLIA,<sup>579</sup> the range of acceptable performance is reduced to 3.3 to 6.8 [ $X+1.5$  to  $X+5.0$ ] mmol/L for the ABL800 and 2.91 to 21.91 [ $X+1.0$  to  $X+20.0$ ] mmol/L for the Cobas400. As such, if either the ABL800 or the Cobas400 is used to validate another analyzer, consideration must be given to desired reliability and the range of interest as performance may not be sufficient at all measurable concentrations. Further work is necessary to clarify the most appropriate consensus-based allowable total error limits for lactate. In light of the results of this study, however, a total error requirement of 15% appears more suitable than the current ASVCP requirement of 40%.

Equivalency of the ABL800 and Cobas400 for the measurement of lactate in canine plasma has not previously been published. One study in people, however, compared a similar analyzer, the Radiometer ABL 90 (Radiometer, Bronshoj Denmark), against the mean of the Cobas Integra 400 and a second analyzer, the Vitros 350 (Ortho Clinical Diagnostics, Rochester NY), as a combined nominated reference method.<sup>583</sup> These authors found that within the physiologic reference interval ( $\leq 2.3$  mmol/L), 97% of values measured on the ABL90 fell within 0.3 mmol/L of the mean of the Cobas400 and the Vitros 350. At values  $\geq 2.3$  mmol/L, however, only 60% of measured values fell within 10% of the concentration reported by the Cobas400/Vitros350. In addition, there was little bias observed  $< 6$  mmol/L, but a substantial negative bias was found at concentrations  $> 6$  mmol/L. These results are similar to the findings of our study, in that agreement was best at low concentrations and a progressive negative proportional bias was identified as concentrations increased.

In the method comparison study, the Bland-Altman plot revealed that the two analyzers cannot be considered equivalent as the ABL800 had a negative constant and proportional bias compared to the Cobas400. The difference between analyzers was, however, small enough to be unlikely to affect overall clinical decision making apart from at very specific clinical decision points. (Tables 2 and 3) For example, if the upper end of the reference interval (roughly 2.5 mmol/L) is used to stratify patients into those at greater risk of death or complications, a difference of 0.1 mmol/L would be required to result in one analyzer classifying a patient as at risk and the other as not at risk. As at  $X+0.5$  (ABL800: 2.32 mmol/L, Cobas400: 2.41 mmol/L) and  $X+1.0$  (ABL800: 2.82 mmol/L, Cobas400: 2.91 mmol/L) the difference between analyzers was only 0.03 and 0.01 mmol/L, respectively, it is likely that the two analyzers would classify a patient within the same category. In contrast, if 6.0 mmol/L is used as a clinical decision making point (e.g. when prognosticating for GDV,<sup>110,384</sup> it is possible that the same patient could be categorized as at risk by the Cobas400 (i.e. 6.2 mmol/L) but not at risk by the ABL800 (i.e. 5.9 mmol/L) because at  $X+4.0$  (ABL800: 5.8 mmol/L, Cobas400: 5.9 mmol/L) the difference between methods was 0.31 mmol/L. This is further complicated by the fact that total error also influences every measurement. Based on calculated total error at  $X+4.0$ , a patient with a true lactate of 6.0 mmol/L could produce a result varying by up to 0.4 mmol/L on either analyzer. Given the presence of a small amount of constant and proportional bias, reference intervals should ideally be established for each analyzer. In addition, patient specific trending should be performed on the same instrument unless appropriately validated correction equations have been established.

While this study satisfied our objectives, it is not without limitations. As a gold standard method for measuring lactate in canine plasma has not been established, we elected to evaluate accuracy on two lactate analyzers via spike and recovery testing with certified reference standard sodium lactate. A certified reference material is considered the next best representation of a “true” analyte quantity.<sup>552</sup> The use of a reference standard can be associated with commutability issues, referring to whether the reference material interacts with the test system similarly to a patient sample.<sup>552</sup> We attempted to

address this concern by replicating the usual patient medium as closely as possible by using canine plasma as our test medium.

The Cobas400 is designed to measure lactate concentration in separated plasma. As such, spiked canine plasma is the ideal matrix for this analyzer. Although heparinized whole blood is the typical test substrate for the ABL800, red blood cells are not hemolyzed during analysis and, as such, it is in fact plasma lactate, rather than true whole blood lactate, that is being measured and reported. Consequently, spiked canine plasma is also a suitable test substrate for the ABL800.

An alternative approach would have been to use blood samples collected from a population of dogs presented to our hospital. The vast majority of patients presenting to a veterinary hospital, however, will have low lactate concentrations. As such, gathering a sufficiently distributed spread of measurements encompassing a wide enough range to satisfy validation requirements would not have been easily achieved. In addition, because lactate is produced and metabolized by red and white blood cells, whole blood processing would have introduced error to the amount of lactate in each sample. While lactate could be processed on the ABL800 immediately after sample collection, the Cobas400 requires centrifugation and separation of plasma prior to analysis. This delay could be significant as lactate concentrations in heparinized whole blood have been shown to increase by >20% over 15 minutes at room temperature.<sup>516</sup> In addition, for precision analysis 20 replicates are required. On the ABL800 it would have taken roughly 40 minutes to process 20 replicates; enough time for the cellular fraction to influence lactate concentrations. Although the glycolytic inhibitor sodium fluoride could have been used to stop lactate production, it does not arrest the monocarboxylate transporters on red blood cell membranes and as such would not stop erythrocytes from taking up lactate from the surrounding plasma.<sup>56</sup> Furthermore, Radiometer, the manufacturer of the ABL800, reports that sodium fluoride can damage the lactate electrode and can cause erroneously high lactate measurements.<sup>586</sup> Radiometer advises the exclusive use of either lithium or sodium heparin as an anticoagulant for samples processed on the ABL800.<sup>586</sup>

All canine plasma inevitably contains a physiologic concentration of lactate. While we minimized individual variability by pooling plasma collected from 5 dogs, we were unable to investigate the lower limit of detection or quantification in this study. Although lactate spiked saline could have been used to avoid this issue, it would not have allowed evaluation of potential matrix effects associated with canine plasma. For example, in a benchtop study evaluating the analytical performance of a similar analyzer to the ABL800, the Radiometer EML 105, lactate concentrations were consistently underestimated in the absence of albumin, whereas when albumin was present lactate was overestimated at low concentrations and underestimated at high concentrations.<sup>595</sup> The ASVCP also suggests that analyte solutions with matrices approximating real samples are preferred to saline dilution during method validation.<sup>578</sup> Although we were unable to evaluate lower limit of detection or quantification, low lactate concentrations have no clinical significance. Further pursuit of this variable would only have been to satisfy academic curiosity. This is

supported by the fact that the ASVCP has not assigned a TE<sub>a</sub> threshold for low lactate levels, deeming it not clinically relevant.<sup>579</sup>

We attempted to mitigate the impact of endogenous lactate by subtracting the analyzer-specific baseline lactate concentration from the measured concentrations for accuracy and method comparison analyses, essentially isolating the known spiked concentration. At 0.25 mmol/L, the final product after seven 50% stepwise dilutions (Appendix C), measured lactate was within 0.05 mmol/L of the expected concentration with SD less than 0.05 mmol/L on both analyzers. Consequently, we feel optimistic that the uncontrolled, endogenous lactate in our test medium did not meaningfully influence results, as we would have anticipated our final dilution to be further from the intended concentration if that had been the case. Furthermore, any potential influence should have decreased as spiked lactate concentration increased relative to basal concentration.

We elected to use total measured lactate (baseline + spiked [lactate]) for linearity and precision analysis. In contrast, we elected to use only the spiked component for accuracy and method comparison testing. As evaluation of precision and linearity does not require perfect accuracy, we were able to evaluate these qualities across the true tested range. In contrast, to maintain scientific rigor, accuracy was evaluated by removing the uncontrolled variable, baseline lactate, from analysis. Similarly, for equivalency testing, direct method comparison would not have been possible without exclusion of the baseline concentrations.

During method comparison, it is recommended that samples be analyzed on both analyzers within 2 hours of one another.<sup>578</sup> This was not feasible in our study because unlike the Cobas400, the ABL800 cannot process batched samples. Each sample on the ABL800 takes approximately 2 minutes to process. Additionally, quality control activities are automatic and interrupt sample analysis causing further delay. That being said, all sample processing on both analyzers using the spiked canine plasma was completed within the same 20 hour period.

We elected not to perform inter-assay precision analysis using spiked canine plasma. Excellent inter-assay precision was established with manufacturer controls. In addition, intra-assay precision using spiked plasma was comparable on the ABL800 and better on the Cobas400 than intra-assay precision using control solutions. It was felt that inter-assay precision analysis using spiked canine plasma was unlikely to provide sufficient additional information to be worth the additional time and expense, particularly as extended sample stability testing would have also been required. This could, however, be an area for future research. We also elected not to evaluate interfering substances in this study as interferents have already been investigated by the manufacturers<sup>584,587</sup> and significant species differences were considered unlikely, albeit not impossible.

Importantly, analytical performance is not the only characteristic that should be considered when selecting an analyzer for clinical use. Other factors such as cost, sample

volume, analyzer footprint, ease of use, and time to result warrant consideration. Both analyzers used in this study require small sample volumes and are relatively easy to use with adequate training. The ABL800 offers the added clinically relevant benefits of a smaller footprint and rapid sample analysis time without the need to separate plasma, making rapid bedside measurement feasible.

With regards to use in clinical research, the relatively generous ASVCP TE<sub>a</sub> of 40% could allow for the acquisition of unreliable data. The more stringent CLIA limit of 10% is more appropriate for research endeavors, resulting in a range of acceptable analytical performance of 3.3 to 6.8 (X+1.5 to X+5.0) mmol/L for the ABL800 and 2.91 to 21.91 (X+1.0 to X+20.0) mmol/L for the Cobas400. Preferably, in a research environment, replicate samples would be analyzed. This would minimize the impact of imprecision making accuracy the primary source of potential analytical error. SE was minimal ( $\leq 8\%$ ) between 3.41 and 21.91 (X+1.5 to X+20.0) mmol/L on the Cobas400 and between 2.3 and 6.8 (X+0.5 to X+5.0) mmol/L on the ABL800. Depending on the researcher's accuracy requirements, however, either analyzer may be considered acceptable as SE was  $\leq 15\%$  at all but the most extreme concentration levels.

Now that analytical performance has been established for the ABL800 and Cobas400, additional research is warranted to determine analyzer and species-specific reference intervals and evaluate diagnostic performance (i.e. sensitivity, specificity, predictive values, etc.). In addition, these analyzers can now be used as reference methods for the validation of other analyzers being used to measure lactate in dogs. Additional research is, however, still required to establish the analytical performance of the ABL800 and Cobas400 for use in other species.

## CONCLUSION

This single-laboratory study partially validated two commercially available analyzers for the measurement of lactate in dogs. Both analyzers demonstrated excellent precision at all tested concentrations. The ABL800 demonstrated excellent accuracy within a narrow, particularly clinically relevant concentration range, whereas the Cobas400 demonstrated overall superior accuracy across a wider range of concentrations. Based on this study, using accuracy and precision limits based on biological variation, the reportable range for the ABL 800 was 2.3 to 6.8 (X+0.5 to X+5.0) mmol/L and was 3.41 to 21.91 mmol/L (X+1.5 to X+20.0 mmol/L) for the Cobas400. Method comparison analysis revealed that while the ABL800 can be considered acceptable when compared to the Cobas400, the analyzers cannot be considered equivalent due to the presence of a small but clinically relevant amount of constant and proportional bias. Both analyzers demonstrated sufficient analytical performance to be applied in clinical practice, although analyzer specific reference intervals have yet to be established. In addition, either analyzer can now be used as a reference method for future validation studies.

## SUMMATING CONCLUSION

An extensive literature review revealed that the traditional understanding of lactate as a simple marker of anaerobic metabolism is flawed. On the contrary, lactate is an important energy storage molecule, the production of which mitigates acidosis resulting from ATP hydrolysis when mitochondrial proton consumption is compromised. The causes of hyperlactatemia (which may occur under aerobic or anaerobic conditions) are highly varied in not only etiology, but also cellular mechanism and clinical significance.

Both the human medical and veterinary literature clearly demonstrate that increased lactate concentration is associated with increased morbidity and mortality. In appropriately selected populations, increased lactate, particularly persistently increased lactate, is moderately predictive in identifying non-survivors. In addition, a normal lactate concentration often predicts survival. Consequently, lactate is a valuable triage and risk stratification tool that can be used to separate patients into higher and lower risk categories. Because hyperlactatemia is a protective response, treatment should be directed at the underlying cause.

Lactate can be measured in whole blood or plasma and from different sample sites, however the same sample type and sample site should be used when measuring lactate serially in an individual patient. Consideration of pre-analytical variables is important, as sampling technique, time to analysis, and collection tube type can influence results. Reference intervals differ between analyzers and consequently, results cannot be directly compared between methods.

Method validation is necessary before a new analyzer is used in clinical practice. An evaluation of analytical performance is the first stage of method validation. Although a number of benchtop analyzers have been used to validate hand-held lactate analyzers, no studies have been performed to validate the use of benchtop analyzers for the measurement of lactate in dogs. As such, we performed a partial method validation study to evaluate the analytical performance and equivalency of two benchtop lactate analyzers in dogs. We investigated linearity, precision, accuracy via recovery, range and equivalency of the ABL800, a high-quality, moderate-volume, point-of-care benchtop analyzer, and the Cobas400, a high-quality, high-volume benchtop clinical laboratory analyzer. Pooled, heparinized canine plasma spiked with known concentrations of reference standard sodium lactate was used as the test medium.

Both analyzers satisfied total error requirements pre-specified by the ASVCP at all tested concentrations.<sup>579</sup> Precision was excellent for both analyzers at all levels. Within a narrower but highly clinically significant range, the ABL800 demonstrated excellent accuracy. The Cobas 400, however, was sufficiently accurate over a wider range of concentrations. Based on acceptance limits of CV < 13.6% and SE < 8%, the reportable range for the ABL 800 was 2.3 to 6.8 (X+0.5 to X+5.0) mmol/L and was 3.41 to 21.91

mmol/L ( $X+1.5$  to  $X+20.0$  mmol/L) for the Cobas400. Method comparison analysis revealed that while the ABL800 can be considered acceptable for clinical use when compared to the Cobas400, the analyzers cannot be considered equivalent due to the presence of a small amount of constant and proportional bias.

In summary, a thorough literature review revealed an important gap in the veterinary literature; an absence of method validation studies evaluating benchtop analyzers for the measurement of lactate in dogs. Consequently, we evaluated the analytical performance of two benchtop analyzers, a high-end point-of-care analyzer and a clinical laboratory analyzer, for the measurement of lactate in canine plasma. Both analyzers demonstrated sufficient analytical performance for use in clinical practice. In addition, both analyzers can now be used as reference methods to validate other lactate analyzers with a strong scientific foundation.

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## **APPENDICES**

### **APPENDIX 1**

Rosenstein PG, Tennent-Brown BS, Hughes D. State of the Art Review: Clinical use of plasma lactate concentration (Part 1): Physiology, pathophysiology and measurement. J Vet Emerg Crit Care 2018; 28 (2): 85-105.

<https://onlinelibrary.wiley.com/doi/10.1111/vec.12708>

## **APPENDIX 2**

Rosenstein PG, Tennent-Brown BS, Hughes D. State of the Art Review: Clinical use of plasma lactate concentration (Part 2): Prognostic and diagnostic utility and the clinical management of hyperlactatemia. J Vet Emerg Crit Care 2018; 28 (2): 106-121.

<https://onlinelibrary.wiley.com/doi/10.1111/vec.12706>

## APPENDIX 3

### Preparation of Stepwise Dilutions

#### *Relevant conversion factors and other information*

Molar weight sodium lactate (NaLac,  $\text{NaC}_3\text{H}_5\text{O}_3$ ) = 112.05977 g/mol

Molar weight sodium<sup>+</sup> ( $\text{Na}^+$ ) = 22.989770 g/mol

Molar weight lactate<sup>-</sup> ( $\text{Lac}^-$ ,  $\text{C}_3\text{H}_5\text{O}_3^-$ ) = 89.069587 g/mol

% lactate<sup>-</sup> =  $89.069587 / 112.05977 \times 100\% = 0.79483999476351 \times 100\% = 79.48\%$

%  $\text{Na}^+$  =  $(1 - 0.79483999476351) \times 100\% = 0.20516000523649 \times 100\% = 20.52\%$

1 mg/dL = 0.112 mmol/L of lactate

1 mmol/L = 9.009 mg/dL of lactate

All dilutions were performed on 31/08/2015

A different pipette was used when interacting with base plasma and for each series of dilutions to prevent cross-contamination

X = Baseline lactate; the concentration of endogenous lactate found in the unspiked, pooled plasma

#### *Dilution series A*

Calculation 1:

Target starting concentration: 32 mmol/L lactate<sup>-</sup>

Starting volume of plasma: 16 ml

32 mmol/L lactate<sup>-</sup>

=  $0.032 \text{ mol/L} \times 89.069587 \text{ g/mol}$

= 2.850226784 g/L

=  $2850.226784 \text{ mg/L} \times 1\text{L}/1000 \text{ mL}$

= 2.850226784 mg/ml [lactate<sup>-</sup>]

$$\begin{aligned}
& [\text{NaLac}] \\
&= 2.850226784 \text{ mg/ml } [\text{lactate}^-] \times 120.516000523649\% \text{ weight NaLac} \\
&= 3.43497932593062 \text{ mg/ml } [\text{NaLac}]
\end{aligned}$$

$$\begin{aligned}
& \text{NaLac mg to add to 16 ml plasma:} \\
& 3.43497932593062 \text{ mg/ml } [\text{NaLac}] \times 16 \text{ ml} \\
&= 54.95966921488992 \text{ mg} \\
&\approx 54.960 \text{ mg}
\end{aligned}$$

$$\therefore 32 \text{ mmol/L } [\text{lactate}^-] \approx 54.960 \text{ mg NaLac} / 16 \text{ mls plasma}$$

Calculation 2:

$$\begin{aligned}
& 32 \text{ mmol/L} \times (1 \text{ mg/1 dL}) \times (1 \text{ L/0.112 mmol}) \\
&= 285.71428571428571 \text{ mg/dL } [\text{Lac}^-] \times 0.01 \text{ mg/ml} \\
&= 2.8571428571428571 \text{ mg/ml } [\text{Lac}^-] \times 120.516000523649\% \text{ weight NaLac} \\
&= 3.44331430067569 \text{ mg/ml } [\text{NaLac}] \times 16 \text{ ml} \\
&= 55.09302881081104 \text{ mg NaLac} / 16 \text{ ml plasma} \\
&\approx 55.093 / 16 \text{ ml plasma}
\end{aligned}$$

$$\therefore 32 \text{ mmol/L } [\text{lactate}^-] \approx 55.093 \text{ mg NaLac} / 16 \text{ mls plasma}$$

$$\therefore \text{Target: } 54.960 - 55.093 \text{ mg} / 16 \text{ mls plasma} \approx 55.0 \text{ mg} / 16 \text{ ml plasma} = 3.4375 \text{ mg/ml}$$

Laboratory Procedure:

1. Weighed 55.2 mg of 100% USP reference standard sodium lactate using precision gram scale
2. Volume plasma required to create 32 mmol/L  $[\text{Lac}^-]$  solution:  
 $55.2 \text{ mg} \times 1 \text{ ml} / 3.4375 \text{ mg} = 16.058188 \text{ ml}$
3. Labelled 8 cylinders A1 – A8
4. Pipetted 8 ml pooled canine plasma into each of A1-A8
5. Added 55.2 mg NaLac into A1
6. Pipetted 8 ml additional plasma into A1
7. Used micropipette to add 0.058 ml into A1
8. Using new pipette, mixed A1 five times and visually inspected to ensure uniform solution without visible crystalline material
9. Transferred 8 ml from A1 into A2 and mixed five times
10. Transferred 8 ml from A2 into A3 and mixed five times
11. Transferred 8 ml from A3 into A4 and mixed five times
12. Transferred 8 ml from A4 into A5 and mixed five times
13. Transferred 8 ml from A5 into A6 and mixed five times

14. Transferred 8 ml from A6 into A7 and mixed five times
15. Transferred 8 ml from A7 into A8 and mixed five times
16. Capped all 8 collection cylinders and placed in fridge at 4°C

Final concentrations [Lac<sup>-</sup>]:

$$A1 = 32 \text{ mmol/L} + x$$

$$A2 = 16 \text{ mmol/L} + x$$

$$A3 = 8 \text{ mmol/L} + x$$

$$A4 = 4 \text{ mmol/L} + x$$

$$A5 = 2 \text{ mmol/L} + x$$

$$A6 = 1 \text{ mmol/L} + x$$

$$A7 = 0.5 \text{ mmol/L} + x$$

$$A8 = 0.25 \text{ mmol/L} + x$$

Where  $x$  = baseline [lactate] in plasma prior to supplementation

#### *Dilution series B*

Calculation 1:

Target starting concentration: 40 mmol/L lactate<sup>-</sup>

Starting volume of plasma: 16 ml

40 mmol/L lactate<sup>-</sup>

$$= 0.040 \text{ mol/L} \times 89.069587 \text{ g/mol}$$

$$= 3.56278348 \text{ g/L}$$

$$= 3562.78348 \text{ mg/L} \times 1\text{L}/1000 \text{ mL}$$

$$= 3.56278348 \text{ mg/ml} [\text{lactate}^-]$$

[NaLac]

$$= 3.56278348 \text{ mg/ml} [\text{lactate}^-] \times 120.516000523649\% \text{ weight NaLac}$$

$$= 4.29372415741328 \text{ mg/ml} [\text{NaLac}]$$

NaLac mg to add to 16 ml plasma:

$$4.29372415741328 \text{ mg/ml} [\text{NaLac}] \times 16 \text{ ml}$$

$$= 68.69958651861248 \text{ mg}$$

$$\approx 68.700 \text{ mg}$$

$$\therefore 40 \text{ mmol/L} [\text{lactate}^-] \approx 68.700 \text{ mg NaLac} / 16 \text{ mls plasma}$$

Calculation 2:

$$40 \text{ mmol/L} \times (1 \text{ mg}/1 \text{ dL}) \times (1 \text{ L}/0.112 \text{ mmol})$$

$$= 357.14285714285714 \text{ mg/dL} [\text{Lac}^-] \times 0.01 \text{ mg/ml}$$

$= 3.5714285714285714 \text{ mg/ml [Lac}^-] \times 120.516000523649\% \text{ weight NaLac}$   
 $= 4.30414287584461 \text{ mg/ml [NaLac]} \times 16 \text{ ml}$   
 $= 68.86628601351376 \text{ mg NaLac} / 16 \text{ ml plasma}$   
 $\approx 68.866 \text{ mg NaLac} / 16 \text{ ml plasma}$

$\therefore 32 \text{ mmol/L [lactate}^-] \approx 68.866 \text{ mg NaLac} / 16 \text{ mls plasma}$

$\therefore \text{Target: } 68.700 - 68.866 \text{ mg} / 16 \text{ mls plasma} \approx 68.8 \text{ mg} / 16 \text{ ml plasma} = 4.3 \text{ mg/ml}$

#### Laboratory Procedure:

1. Weighed 69.7 mg of 100% USP reference standard sodium lactate using precision gram scale
2. Volume plasma required to create 40 mmol/L [Lac<sup>-</sup>] solution:  
 $69.7 \text{ mg} \times 1 \text{ ml} / 4.3 \text{ mg} = 16.2 \text{ ml}$
3. Labelled 5 cylinders B1 – B5
4. Pipetted 8 ml pooled canine plasma into each of B1-B5
5. Added 69.7 mg NaLac into B1
6. Pipetted 8 ml additional plasma into B1
7. Used micropipette to add 0.2 ml into B1
8. Using new pipette, mixed B1 five times and visually inspected to ensure uniform solution without visible crystalline material
9. Transferred 8 ml from B1 into B2 and mixed five times
10. Transferred 8 ml from B2 into B3 and mixed five times
11. Transferred 8 ml from B3 into B4 and mixed five times
12. Transferred 8 ml from B4 into B5 and mixed five times
13. Capped all 5 collection cylinders and placed in fridge at 4°C

Final concentrations [Lac<sup>-</sup>]:

B1 = 40 mmol/L + x

B2 = 20 mmol/L + x

B3 = 10 mmol/L + x

B4 = 5 mmol/L + x

B5 = 2.5 mmol/L + x

#### *Dilution series C*

Calculation 1:

Target starting concentration: 24 mmol/L lactate<sup>-</sup>

Starting volume of plasma: 16 ml

24 mmol/L lactate<sup>-</sup>

$= 0.024 \text{ mol/L} \times 89.069587 \text{ g/mol}$

$$\begin{aligned}
 &= 2.137670088 \text{ g/L} \\
 &= 2137.670088 \text{ mg/L} \times 1\text{L}/1000 \text{ mL} \\
 &= 2.137670088 \text{ mg/ml [lactate}^-]
 \end{aligned}$$

$$\begin{aligned}
 &[\text{NaLac}] \\
 &= 2.137670088 \text{ mg/ml [lactate}^-] \times 120.516000523649\% \text{ weight NaLac} \\
 &= 2.57623449444797 \text{ mg/ml [NaLac]}
 \end{aligned}$$

$$\begin{aligned}
 &\text{NaLac mg to add to 16 ml plasma:} \\
 &2.57623449444797 \text{ mg/ml [NaLac]} \times 16 \text{ ml} \\
 &= 41.21975191116752 \text{ mg} \\
 &\approx 41.220 \text{ mg}
 \end{aligned}$$

$$\therefore 24 \text{ mmol/L [lactate}^-] \approx 41.220 \text{ mg NaLac} / 16 \text{ mls plasma}$$

Calculation 2:

$$\begin{aligned}
 &24 \text{ mmol/L} \times (1 \text{ mg}/1 \text{ dL}) \times (1 \text{ L}/0.112 \text{ mmol}) \\
 &= 214.28571428571429 \text{ mg/dL [Lac}^-] \times 0.01 \text{ mg/ml} \\
 &= 2.1428571428571429 \text{ mg/ml [Lac}^-] \times 120.516000523649\% \text{ weight NaLac} \\
 &= 2.58248572550676 \text{ mg/ml [NaLac]} \times 16 \text{ ml} \\
 &= 41.31977160810816 \text{ mg NaLac} / 16 \text{ ml plasma} \\
 &\approx 41.320 \text{ mg NaLac} / 16 \text{ ml plasma}
 \end{aligned}$$

$$\therefore 24 \text{ mmol/L [lactate}^-] \approx 41.320 \text{ mg NaLac} / 16 \text{ mls plasma}$$

$$\therefore \text{Target: } 41.220 - 41.320 \text{ mg} / 16 \text{ mls plasma} \approx 41.3 \text{ mg} / 16 \text{ ml plasma} = 2.58125 \text{ mg/ml}$$

Laboratory Procedure:

1. Weighed 43.1 mg of 100% USP reference standard sodium lactate using precision gram scale
2. Volume plasma required to create 24 mmol/L [Lac<sup>-</sup>] solution:  
43.1 mg x 1 ml / 2.58125 mg = 16.6973 ml
3. Labelled 5 cylinders C1 – C5
4. Pipetted 8 ml pooled canine plasma into each of C1-C5
5. Added 43.1 mg NaLac into C1
6. Pipetted 8 ml additional plasma into C1
7. Used micropipette to add 0.697 ml into C1
8. Using new pipette, mixed C1 five times and visually inspected to ensure uniform solution without visible crystalline material
9. Transferred 8 ml from C1 into C2 and mixed five times
10. Transferred 8 ml from C2 into C3 and mixed five times
11. Transferred 8 ml from C3 into C4 and mixed five times

12. Transferred 8 ml from C4 into C5 and mixed five times
13. Capped all 5 collection cylinders and placed in fridge at 4°C

Final concentrations [Lac<sup>-</sup>]:

$$C1 = 24 \text{ mmol/L} + x$$

$$C2 = 12 \text{ mmol/L} + x$$

$$C3 = 6 \text{ mmol/L} + x$$

$$C4 = 3 \text{ mmol/L} + x$$

$$C5 = 1.5 \text{ mmol/L} + x$$

#### *Dilution series D*

Calculation 1:

Target starting concentration: 28 mmol/L lactate<sup>-</sup>

Starting volume of plasma: 16 ml

28 mmol/L lactate<sup>-</sup>

$$= 0.028 \text{ mol/L} \times 89.069587 \text{ g/mol}$$

$$= 2.493948436 \text{ g/L}$$

$$= 2493.948436 \text{ mg/L} \times 1\text{L}/1000 \text{ mL}$$

$$= 2.493948436 \text{ mg/ml} [\text{lactate}^-]$$

[NaLac]

$$= 2.493948436 \text{ mg/ml} [\text{lactate}^-] \times 120.516000523649\% \text{ weight NaLac}$$

$$= 3.00056069101893 \text{ mg/ml} [\text{NaLac}]$$

NaLac mg to add to 16 ml plasma:

$$3.00056069101893 \text{ mg/ml} [\text{NaLac}] \times 16 \text{ ml}$$

$$= 48.0897105630288 \text{ mg}$$

$$\approx 48.100 \text{ mg}$$

$$\therefore 28 \text{ mmol/L} [\text{lactate}^-] \approx 48.100 \text{ mg NaLac} / 16 \text{ mls plasma}$$

Calculation 2:

$$28 \text{ mmol/L} \times (1 \text{ mg}/1 \text{ dL}) \times (1 \text{ L}/0.112 \text{ mmol})$$

$$= 250 \text{ mg/dL} [\text{Lac}^-] \times 0.01 \text{ mg/ml}$$

$$= 2.50 \text{ mg/ml} [\text{Lac}^-] \times 120.516000523649\% \text{ weight NaLac}$$

$$= 3.012900013091225 \text{ mg/ml} [\text{NaLac}] \times 16 \text{ ml}$$

$$= 48.20640020945968 \text{ mg NaLac} / 16 \text{ ml plasma}$$

$$\approx 48.206 \text{ mg NaLac} / 16 \text{ ml plasma}$$

$\therefore$  28 mmol/L [lactate<sup>-</sup>]  $\approx$  48.206 mg NaLac / 16 mls plasma

$\therefore$  Target: 48.100 – 48.206 mg / 16 mls plasma  $\approx$  48.2 mg / 16 ml plasma = 3.0125 mg/ml

Laboratory Procedure:

1. Weighed 49.3 mg of 100% USP reference standard sodium lactate using precision gram scale
2. Volume plasma required to create 28 mmol/L [Lac<sup>-</sup>] solution:  
 $49.3 \text{ mg} \times 1 \text{ ml} / 3.0125 \text{ mg} = 16.36514 \text{ ml}$
3. Labelled 5 cylinders D1 – D4
4. Pipetted 8 ml pooled canine plasma into each of D1 – D4
5. Added 49.3 mg NaLac into D1
6. Pipetted 8 ml additional plasma into D2
7. Used micropipette to add 0.365 ml into D1
8. Using new pipette, mixed D1 five times and visually inspected to ensure uniform solution without visible crystalline material
9. Transferred 8 ml from D1 into D2 and mixed five times
10. Transferred 8 ml from D2 into D3 and mixed five times
11. Transferred 8 ml from D3 into D4 and mixed five times
12. Capped all 4 collection cylinders and placed in fridge at 4°C

Final concentrations [Lac<sup>-</sup>]:

D1 = 28 mmol/L + x

D2 = 14 mmol/L + x

D3 = 7 mmol/L + x

D4 = 3.5 mmol/L + x

## APPENDIX 4

### Linearity using Manufacturer Controls

#### Data for Cobas400

| Expected [Lactate] (mmol/L) | Mean Measured [Lactate] (mmol/L) |
|-----------------------------|----------------------------------|
| 1.6                         | 2.01                             |
| 4.5                         | 5.19                             |
| 10.5                        | 12.18                            |

#### Data for ABL800

| Expected [Lactate] (mmol/L) | Mean Measured [Lactate] (mmol/L) |
|-----------------------------|----------------------------------|
| 1.6                         | 1.74                             |
| 4.5                         | 4.56                             |
| 10.5                        | 10.74                            |

NB: See Appendix L for determination of Mean Measured [Lactate] (mmol/L)

## APPENDIX 5

### Intra-assay Precision using Manufacturer Controls

Data for ABL800

| Replicate Number | Manufacturer Control [Lactate] (mmol/L) |      |       |       |
|------------------|-----------------------------------------|------|-------|-------|
|                  | 1.6                                     | 4.5  | 10.5  | 27    |
| 1                | 1.7                                     | 4.4  | 10.4  | 28    |
| 2                | 1.7                                     | 4.5  | 10.7  | 28    |
| 3                | 1.7                                     | 4.4  | 10.6  | 29    |
| 4                | 1.7                                     | 4.4  | 10.3  | 28    |
| 5                | 1.7                                     | 4.5  | 10.4  | 28    |
| 6                | 1.7                                     | 4.7  | 10.6  | 28    |
| 7                | 1.7                                     | 4.6  | 10.5  | 28    |
| 8                | 1.8                                     | 4.4  | 10.8  | 28    |
| 9                | 1.8                                     | 4.7  | 10.8  | 28    |
| 10               | 1.8                                     | 4.5  | 10.8  | 29    |
| 11               | 1.7                                     | 4.5  | 10.6  | 29    |
| 12               | 1.8                                     | 4.6  | 10.6  | 29    |
| 13               | 1.7                                     | 4.6  | 11    | 28    |
| 14               | 1.7                                     | 4.6  | 10.9  | 29    |
| 15               | 1.8                                     | 4.6  | 10.7  | 29    |
| 16               | 1.7                                     | 4.6  | 10.8  | 28    |
| 17               | 1.7                                     | 4.7  | 10.9  | 29    |
| 18               | 1.8                                     | 4.4  | 11.1  | 29    |
| 19               | 1.8                                     | 4.6  | 11.1  | 29    |
| 20               | 1.8                                     | 4.8  | 11.2  | 29    |
| Mean             | 1.74                                    | 4.56 | 10.74 | 28.50 |
| SD               | 0.05                                    | 0.12 | 0.25  | 0.51  |
| CV%              | 2.9                                     | 2.6  | 2.3   | 1.8   |

Data for Cobas400

| Replicate Number | Manufacturer Control [Lactate] (mmol/L) |      |       |       |
|------------------|-----------------------------------------|------|-------|-------|
|                  | 1.6                                     | 4.5  | 10.5  | 27    |
| 1                | 2.08                                    | 5.2  | 12.35 | 24.1  |
| 2                | 2.08                                    | 5.19 | 12.31 | 24.37 |
| 3                | 2.06                                    | 5.2  | 12.3  | 24.61 |
| 4                | 2.06                                    | 5.2  | 12.23 | 24.26 |
| 5                | 2.08                                    | 5.23 | 12.19 | 24.51 |
| 6                | 2.07                                    | 5.19 | 12.26 | 23.75 |
| 7                | 2.07                                    | 5.19 | 12.2  | 23.83 |
| 8                | 2.08                                    | 5.19 | 12.18 | 23.7  |
| 9                | 2.06                                    | 5.19 | 12.14 | 23.57 |
| 10               | 2.08                                    | 5.16 | 12.12 | 23.53 |
| 11               | 2.05                                    | 5.2  | 12.15 | 23.82 |
| 12               | 2.07                                    | 5.22 | 12.12 | 24.1  |
| 13               | 2.05                                    | 5.18 | 12.15 | 24.22 |
| 14               | 2.07                                    | 5.17 | 12.22 | 24.22 |
| 15               | 2.05                                    | 5.17 | 12.14 | 23.81 |
| 16               | 2.06                                    | 5.18 | 12.18 | 23.9  |
| 17               | 2.07                                    | 5.18 | 12.14 | 24.29 |
| 18               | 2.06                                    | 5.17 | 12.14 | 24.12 |
| 19               | 2.06                                    | 5.22 | 12.09 | 24.56 |
| 20               | 2.06                                    | 5.17 | 12.07 | 24    |
| Mean             | 2.07                                    | 5.19 | 12.18 | 24.06 |
| SD               | 0.01                                    | 0.02 | 0.07  | 0.32  |
| CV%              | 0.5                                     | 0.4  | 0.6   | 1.3   |

## APPENDIX 6

### Inter-assay Precision using Manufacturer Controls

Data for ABL800

| Day  | Manufacturer Control<br>[Lactate] (mmol/L) |      |       |       |
|------|--------------------------------------------|------|-------|-------|
|      | 1.6                                        | 4.5  | 10.5  | 27    |
| 1    | 1.7                                        | 4.4  | 10.4  | 28    |
| 2    | 1.7                                        | 4.4  | 10.5  | 28    |
| 3    | 1.7                                        | 4.4  | 10.5  | 30    |
| 4    | 1.6                                        | 4.4  | 10.5  | 29    |
| 5    | 1.7                                        | 4.4  | 10.5  | 29    |
| 6    | 1.7                                        | 4.6  | 10.7  | 29    |
| 7    | 1.6                                        | 4.4  | 10.3  | 29    |
| 8    | 1.7                                        | 4.5  | 10.5  | 29    |
| 9    | 1.7                                        | 4.5  | 10.4  | 30    |
| 10   | 1.6                                        | 4.5  | 10.3  | 31    |
| 11   | 1.5                                        | 4.5  | 10.4  | 31    |
| 12   | 1.7                                        | 4.5  | 9.9   | 26    |
| 13   | 1.7                                        | 4.4  | 10.1  | 27    |
| 14   | 1.7                                        | 4.5  | 10.3  | 29    |
| 15   | 1.6                                        | 4.7  | 10.3  | 31    |
| 16   | 1.6                                        | 4.8  | 10.3  | 31    |
| 17   | 1.7                                        | 4.8  | 10.6  | 31    |
| 18   | 1.6                                        | 4.8  | 10.9  | 31    |
| 19   | 1.6                                        | 4.6  | 10.7  | 31    |
| 20   | 1.8                                        | 4.7  | 10.1  | 27    |
| Mean | 1.66                                       | 4.54 | 10.41 | 29.35 |
| SD   | 0.07                                       | 0.15 | 0.23  | 1.56  |
| CV%  | 4.1                                        | 3.2  | 2.2   | 5.3   |

Data for Cobas400

| Day  | Manufacturer Control<br>[Lactate] (mmol/L) |      |       |       |
|------|--------------------------------------------|------|-------|-------|
|      | 1.6                                        | 4.5  | 10.5  | 27    |
| 1    | 2.08                                       | 5.2  | 12.35 | 24.1  |
| 2    | 2.09                                       | 5.15 | 12.24 | 24.25 |
| 3    | 2.02                                       | 5.17 | 12.15 | 24.68 |
| 4    | 2.09                                       | 5.28 | 12.44 | 24.13 |
| 5    | 2.06                                       | 5.1  | 12.23 | 23.49 |
| 6    | 2.07                                       | 5.21 | 12.2  | 24.63 |
| 7    | 2.07                                       | 5.22 | 12.37 | 22.05 |
| 8    | 2.06                                       | 5.2  | 12.18 | 24.28 |
| 9    | 2.04                                       | 5.27 | 12.24 | 24.27 |
| 10   | 2.06                                       | 5.14 | 12.1  | 24.39 |
| 11   | 2.1                                        | 5.26 | 12.19 | 23.71 |
| 12   | 2.07                                       | 5.24 | 12.27 | 21.08 |
| 13   | 2.06                                       | 5.1  | 11.97 | 23.04 |
| 14   | 2.05                                       | 5.17 | 12.21 | 24.55 |
| 15   | 2.07                                       | 5.33 | 12.62 | 24.69 |
| 16   | 2.06                                       | 5.23 | 11.94 | 24.13 |
| 17   | 2.09                                       | 5.32 | 12.38 | 23.16 |
| 18   | 2.11                                       | 5.23 | 12.12 | 22.65 |
| 19   | 2.19                                       | 5.38 | 12.64 | 24.46 |
| 20   | 2.19                                       | 5.53 | 12.87 | 24.49 |
| Mean | 2.08                                       | 5.24 | 12.28 | 23.81 |
| SD   | 0.04                                       | 0.1  | 0.22  | 0.97  |
| CV%  | 2.0                                        | 1.9  | 1.8   | 4.1   |

## APPENDIX 7

### Baseline Lactate Concentrations in Spiked Canine Plasma

| Replicate Number | ABL800 | Cobas400 |
|------------------|--------|----------|
| 1                | 1.8    | 1.93     |
| 2                | 1.8    | 1.92     |
| 3                | 1.8    | 1.91     |
| 4                | 1.9    | 1.91     |
| 5                | 1.8    | 1.91     |
| 6                | 1.8    | 1.91     |
| 7                | 1.9    | 1.9      |
| 8                | 1.8    | 1.91     |
| 9                | 1.8    | 1.9      |
| 10               | 1.8    | 1.91     |
| 11               | 1.8    | 1.91     |
| 12               | 1.8    | 1.9      |
| 13               | 1.8    | 1.9      |
| 14               | 1.8    | 1.91     |
| 15               | 1.8    | 1.9      |
| 16               | 1.8    | 1.92     |
| 17               | 1.9    | 1.91     |
| 18               | 1.9    | 1.91     |
| 19               | 1.8    | 1.91     |
| 20               | 1.8    | 1.91     |
| Mean             | 1.82   | 1.91     |
| SD               | 0.04   | 0.01     |
| CV%              | 2.2    | 0.4      |

## APPENDIX 8

### Linearity using Spiked Canine Plasma

Data for ABL800

| Expected [Lactate]<br>(mmol/L) | Mean Measured<br>[Lactate] (mmol/L) |
|--------------------------------|-------------------------------------|
| 2.07                           | 2.12                                |
| 2.32                           | 2.36                                |
| 2.82                           | 2.90                                |
| 3.32                           | 3.35                                |
| 3.82                           | 3.84                                |
| 4.32                           | 4.38                                |
| 4.82                           | 4.78                                |
| 5.32                           | 5.26                                |
| 5.82                           | 5.731                               |
| 6.82                           | 6.74                                |
| 7.82                           | 7.30                                |
| 8.82                           | 8.38                                |
| 9.82                           | 8.97                                |
| 11.82                          | 10.98                               |
| 13.82                          | 12.20                               |
| 21.82                          | 19.40                               |
| 33.82                          | 28.90                               |

Data for Cobas400

| Expected [Lactate]<br>(mmol/L) | Mean Measured<br>[Lactate] (mmol/L) |
|--------------------------------|-------------------------------------|
| 2.1595                         | 2.19                                |
| 2.4095                         | 2.48                                |
| 2.9095                         | 2.996                               |
| 3.4095                         | 3.52                                |
| 3.9095                         | 4.04                                |
| 4.4095                         | 4.59                                |
| 4.9095                         | 5.028                               |
| 5.4095                         | 5.61                                |
| 5.9095                         | 6.13                                |
| 6.9095                         | 7.19                                |
| 7.9095                         | 8.12                                |
| 8.9095                         | 9.21                                |
| 9.9095                         | 10.08                               |
| 11.9095                        | 12.18                               |
| 13.9095                        | 13.97                               |
| 21.9095                        | 21.01                               |
| 33.9095                        | 26.55                               |

NB: See Appendix L for determination of Mean Measured [Lactate] (mmol/L)

## APPENDIX 9: Intra-assay Precision using Spiked Canine Plasma

Data for ABL800

| Replicate Number | Expected [Lactate] (mmol/L); X = 1.82 |        |       |       |       |       |       |       |       |       |       |       |        |        |        |        |
|------------------|---------------------------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|
|                  | X+0                                   | X+0.25 | X+0.5 | X+1.0 | X+1.5 | X+2.0 | X+2.5 | X+3.0 | X+3.5 | X+4.0 | X+5.0 | X+7.0 | X+10.0 | X+16.0 | X+20.0 | X+32.0 |
|                  | N/A                                   | 2.07   | 2.32  | 2.82  | 3.32  | 3.82  | 4.32  | 4.82  | 5.32  | 5.82  | 6.82  | 8.82  | 11.82  | 17.82  | 21.82  | 33.82  |
| 1                | 1.8                                   | 2      | 2.2   | 2.8   | 3.2   | 3.8   | 4.2   | 4.8   | 5.0   | 5.5   | 6.4   | 8.1   | 10.5   | 16     | 18     | 27     |
| 2                | 1.8                                   | 2      | 2.4   | 2.9   | 3.4   | 3.8   | 4.3   | 4.8   | 5.2   | 5.7   | 6.6   | 8.0   | 10.7   | 16     | 19     | 28     |
| 3                | 1.8                                   | 2.1    | 2.3   | 2.9   | 3.4   | 3.8   | 4.4   | 4.8   | 5.3   | 5.6   | 6.6   | 8.3   | 10.9   | 16     | 19     | 29     |
| 4                | 1.9                                   | 2.1    | 2.3   | 3     | 3.4   | 3.9   | 4.4   | 4.7   | 5.3   | 5.5   | 6.5   | 8.4   | 10.8   | 16     | 19     | 29     |
| 5                | 1.8                                   | 2.2    | 2.3   | 2.9   | 3.4   | 3.9   | 4.4   | 4.9   | 5.3   | 5.8   | 6.6   | 8.2   | 10.8   | 16     | 19     | 29     |
| 6                | 1.8                                   | 2.2    | 2.5   | 2.8   | 3.4   | 3.8   | 4.4   | 4.7   | 5.3   | 5.8   | 6.8   | 8.4   | 11.3   | 16     | 19     | 29     |
| 7                | 1.9                                   | 2.1    | 2.3   | 2.9   | 3.3   | 3.9   | 4.5   | 4.8   | 5.2   | 5.7   | 6.6   | 8.2   | 10.9   | 16     | 19     | 29     |
| 8                | 1.8                                   | 2.1    | 2.3   | 2.9   | 3.3   | 4     | 4.3   | 4.7   | 5.2   | 5.9   | 6.7   | 8.4   | 11.2   | 16     | 19     | 29     |
| 9                | 1.8                                   | 2.1    | 2.4   | 2.9   | 3.4   | 3.9   | 4.3   | 4.7   | 5.3   | 5.8   | 6.8   | 8.5   | 10.9   | 16     | 19     | 29     |
| 10               | 1.8                                   | 2.1    | 2.4   | 2.9   | 3.4   | 3.8   | 4.4   | 4.8   | 5.2   | 5.8   | 6.7   | 8.5   | 10.9   | 16     | 20     | 29     |
| 11               | 1.8                                   | 2.2    | 2.4   | 2.9   | 3.3   | 3.9   | 4.4   | 4.8   | 5.2   | 6.0   | 6.7   | 8.4   | 11.5   | 16     | 20     | 29     |
| 12               | 1.8                                   | 2.1    | 2.3   | 2.9   | 3.3   | 3.8   | 4.4   | 4.9   | 5.3   | 5.8   | 6.9   | 8.5   | 10.9   | 16     | 19     | 29     |
| 13               | 1.8                                   | 2.1    | 2.4   | 3.0   | 3.3   | 3.8   | 4.3   | 4.8   | 5.4   | 5.7   | 6.8   | 8.7   | 11.1   | 16     | 20     | 29     |
| 14               | 1.8                                   | 2.1    | 2.4   | 2.9   | 3.4   | 3.8   | 4.5   | 4.8   | 5.3   | 5.7   | 6.8   | 8.5   | 11.4   | 16     | 20     | 29     |
| 15               | 1.8                                   | 2.2    | 2.4   | 2.9   | 3.4   | 3.9   | 4.5   | 4.8   | 5.3   | 5.6   | 6.8   | 8.4   | 11.1   | 16     | 19     | 29     |
| 16               | 1.8                                   | 2.2    | 2.4   | 2.9   | 3.4   | 3.8   | 4.5   | 4.7   | 5.3   | 5.8   | 6.9   | 8.4   | 11.3   | 16     | 20     | 30     |
| 17               | 1.9                                   | 2.2    | 2.4   | 2.9   | 3.4   | 3.8   | 4.3   | 4.8   | 5.3   | *     | 7.0   | 8.3   | 10.8   | 16     | 20     | 30     |
| 18               | 1.9                                   | 2.1    | 2.4   | 2.9   | 3.3   | 3.9   | 4.3   | 4.8   | 5.4   | *     | 6.9   | 8.4   | 11.1   | 16     | 20     | 29     |
| 19               | 1.8                                   | 2.2    | 2.4   | 3.0   | 3.3   | 3.7   | 4.4   | 4.9   | 5.3   | *     | 7.0   | 8.5   | 10.8   | 16     | 20     | 28     |
| 20               | 1.8                                   | 2.1    | 2.4   | 2.9   | 3.3   | 3.7   | 4.3   | 4.7   | 5.1   | *     | 6.7   | 8.4   | 10.8   | 16     | 20     | 29     |
| Mean             | 1.82                                  | 2.12   | 2.36  | 2.90  | 3.35  | 3.84  | 4.38  | 4.78  | 5.26  | 5.73  | 6.74  | 8.38  | 10.98  | 16.00  | 19.40  | 28.90  |
| SD               | 0.04                                  | 0.06   | 0.07  | 0.05  | 0.06  | 0.07  | 0.08  | 0.07  | 0.09  | 0.14  | 0.16  | 0.16  | 0.26   | 0.00   | 0.60   | 0.64   |
| CV%              | 2.2                                   | 3.0    | 2.8   | 1.8   | 1.8   | 1.9   | 1.9   | 1.4   | 1.8   | 2.4   | 2.4   | 1.9   | 2.3    | 0.0    | 3.1    | 2.2    |

\* Sample Error

## APPENDIX 10: Intra-assay Precision using Spiked Canine Plasma

### Data for Cobas400

| Replicate Number | Expected [Lactate] (mmol/L); X = 1.91 |        |       |       |       |       |       |       |       |       |       |       |        |        |        |        |
|------------------|---------------------------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|
|                  | X+0                                   | X+0.25 | X+0.5 | X+1.0 | X+1.5 | X+2.0 | X+2.5 | X+3.0 | X+3.5 | X+4.0 | X+5.0 | X+7.0 | X+10.0 | X+16.0 | X+20.0 | X+32.0 |
|                  | N/A                                   | 2.07   | 2.32  | 2.82  | 3.32  | 3.82  | 4.32  | 4.82  | 5.32  | 5.82  | 6.82  | 8.82  | 11.82  | 17.82  | 21.82  | 33.82  |
| 1                | 1.93                                  | 2.20   | 2.49  | 3.01  | 3.54  | 4.01  | 4.59  | 5.05  | 5.62  | 6.10  | 7.21  | 9.33  | 12.21  | >15.50 | 21.22  | 26.44  |
| 2                | 1.92                                  | 2.18   | 2.48  | 3.01  | 3.52  | 4.03  | 4.59  | 5.04  | 5.61  | 6.14  | 7.15  | 9.25  | 12.23  | >15.50 | 21.23  | 26.27  |
| 3                | 1.91                                  | 2.19   | 2.47  | 3.01  | 3.52  | 4.03  | 4.59  | 5.01  | 5.61  | 6.10  | 7.21  | 9.28  | 12.18  | >15.50 | 21.25  | 26.26  |
| 4                | 1.91                                  | 2.19   | 2.47  | 2.98  | 3.52  | 4.03  | 4.63  | 5.00  | 5.60  | 6.10  | 7.16  | 9.22  | 12.22  | >15.50 | 21.14  | 26.91  |
| 5                | 1.91                                  | 2.19   | 2.47  | 2.99  | 3.53  | 4.02  | 4.59  | 5.04  | 5.60  | 6.12  | 7.16  | 9.30  | 12.14  | >15.50 | 20.92  | 26.68  |
| 6                | 1.91                                  | 2.19   | 2.51  | 2.99  | 3.53  | 4.05  | 4.59  | 5.05  | 5.63  | 6.14  | 7.19  | 9.22  | 12.21  | >15.50 | 21.03  | 26.97  |
| 7                | 1.90                                  | 2.19   | 2.48  | 2.99  | 3.53  | 4.03  | 4.60  | 5.01  | 5.67  | 6.14  | 7.21  | 9.23  | 12.17  | >15.50 | 21.02  | 26.80  |
| 8                | 1.91                                  | 2.19   | 2.46  | 3.00  | 3.52  | 4.07  | 4.59  | 5.03  | 5.63  | 6.13  | 7.22  | 9.20  | 12.07  | >15.50 | 21.05  | 26.68  |
| 9                | 1.90                                  | 2.19   | 2.48  | 2.99  | 3.52  | 4.06  | 4.59  | 5.05  | 5.59  | 6.12  | 7.16  | 9.25  | 12.21  | >15.50 | >15.50 | 27.52  |
| 10               | 1.91                                  | 2.18   | 2.48  | 2.98  | 3.51  | 4.03  | 4.6   | 5.03  | 5.62  | 6.14  | 7.22  | 9.18  | 12.20  | >15.50 | >15.50 | 27.07  |
| 11               | 1.91                                  | 2.19   | 2.48  | 3.00  | 3.52  | 4.03  | 4.58  | 5.02  | 5.60  | 6.13  | 7.23  | 9.23  | 12.18  | >15.50 | 20.77  | 27.04  |
| 12               | 1.90                                  | 2.19   | 2.47  | 3.00  | 3.55  | 4.05  | 4.60  | 5.01  | 5.61  | 6.11  | 7.19  | 9.13  | 12.19  | >15.50 | 20.96  | 26.72  |
| 13               | 1.90                                  | 2.21   | 2.48  | 3.00  | 3.55  | 4.04  | 4.60  | 5.04  | 5.58  | 6.12  | 7.19  | 9.19  | 12.21  | >15.50 | 20.78  | 26.90  |
| 14               | 1.91                                  | 2.21   | 2.48  | 2.99  | 3.51  | 4.03  | 4.59  | 5.02  | 5.63  | 6.15  | 7.20  | 9.23  | 12.16  | >15.50 | 20.92  | 26.79  |
| 15               | 1.90                                  | 2.21   | 2.48  | 2.99  | 3.51  | 4.06  | 4.63  | 5.04  | 5.61  | 6.11  | 7.20  | 9.18  | 12.23  | >15.50 | 20.85  | 26.81  |
| 16               | 1.92                                  | 2.20   | 2.49  | 3.01  | 3.51  | 4.05  | 4.59  | 5.02  | 5.61  | 6.11  | 7.16  | 9.13  | 12.14  | >15.50 | 21.10  | 26.65  |
| 17               | 1.91                                  | 2.20   | 2.46  | 3.01  | 3.51  | 4.03  | 4.60  | 5.02  | 5.60  | 6.15  | 7.19  | 9.19  | 12.19  | >15.50 | >15.50 | 26.25  |
| 18               | 1.91                                  | 2.19   | 2.47  | 2.98  | 3.51  | 4.04  | 4.54  | 5.04  | 5.58  | 6.15  | 7.19  | 9.16  | 12.15  | >15.50 | >15.50 | 25.83  |
| 19               | 1.91                                  | 2.19   | 2.47  | 3.01  | 3.50  | 4.06  | 4.57  | 5.02  | 5.60  | 6.18  | 7.15  | 9.14  | 12.20  | >15.50 | 20.93  | 25.72  |
| 20               | 1.91                                  | 2.20   | 2.47  | 2.99  | 3.50  | 4.06  | 4.55  | 5.02  | 5.64  | 6.12  | 7.24  | 9.20  | 12.18  | >15.50 | 21.01  | 24.78  |
| Mean             | 1.91                                  | 2.19   | 2.48  | 3.00  | 3.52  | 4.04  | 4.59  | 5.03  | 5.61  | 6.13  | 7.19  | 9.21  | 12.18  | Error  | 21.01  | 26.55  |
| SD               | 0.01                                  | 0.01   | 0.01  | 0.01  | 0.01  | 0.02  | 0.02  | 0.02  | 0.02  | 0.02  | 0.03  | 0.05  | 0.04   | N/A    | 0.15   | 0.60   |
| CV%              | 0.4                                   | 0.4    | 0.4   | 0.4   | 0.4   | 0.4   | 0.4   | 0.3   | 0.4   | 0.3   | 0.4   | 0.6   | 0.3    | N/A    | 0.7    | 2.2    |

## APPENDIX 11: Accuracy via Recovery using Spiked Canine Plasma

Data from ABL800

| Number of Replicates | Expected [lactate] | Mean Measured [Lactate] | Mean Measured [Lactate] - X | % Recovery | % Bias |
|----------------------|--------------------|-------------------------|-----------------------------|------------|--------|
| 20                   | 0.25               | 2.12                    | 0.30                        | 122.00     | 22.00  |
| 20                   | 0.50               | 2.36                    | 0.54                        | 109.00     | 9.00   |
| 20                   | 1.00               | 2.90                    | 1.08                        | 108.50     | 8.50   |
| 20                   | 1.50               | 3.35                    | 1.53                        | 102.00     | 2.00   |
| 20                   | 2.00               | 3.84                    | 2.02                        | 100.75     | 0.75   |
| 20                   | 2.50               | 4.38                    | 2.56                        | 102.20     | 2.20   |
| 20                   | 3.00               | 4.78                    | 2.97                        | 98.83      | 1.17   |
| 20                   | 3.50               | 5.26                    | 3.44                        | 98.29      | 1.71   |
| 16                   | 4.00               | 5.73                    | 3.91                        | 97.78      | 2.22   |
| 20                   | 5.00               | 6.74                    | 4.92                        | 98.40      | 1.60   |
| 3                    | 6.00               | 7.30                    | 5.48                        | 91.33      | 8.67   |
| 20                   | 7.00               | 8.38                    | 6.56                        | 93.64      | 6.36   |
| 3                    | 8.00               | 8.97                    | 7.15                        | 89.33      | 10.67  |
| 20                   | 10.00              | 10.98                   | 9.16                        | 91.65      | 8.35   |
| 3                    | 12.00              | 12.20                   | 10.38                       | 86.50      | 13.50  |
| 20                   | 16.00              | 16.00                   | 14.18                       | 88.63      | 11.38  |
| 20                   | 20.00              | 19.40                   | 17.58                       | 87.90      | 12.10  |
| 20                   | 32.00              | 28.90                   | 27.08                       | 84.63      | 15.38  |
| 3                    | 40.00              | High x 3                | N/A                         | N/A        | N/A    |

## APPENDIX 12: Accuracy via Recovery using Spiked Canine Plasma

Data from Cobas400

| Number of Replicates | Expected [lactate] | Mean Measured [Lactate] | Mean Measured [Lactate] - X | % Recovery | % Bias |
|----------------------|--------------------|-------------------------|-----------------------------|------------|--------|
| 20                   | 0.25               | 2.19                    | 0.28                        | 113.80     | 13.80  |
| 20                   | 0.50               | 2.48                    | 0.57                        | 113.50     | 13.50  |
| 20                   | 1.00               | 3.00                    | 1.09                        | 108.70     | 8.70   |
| 20                   | 1.50               | 3.52                    | 1.61                        | 107.40     | 7.40   |
| 20                   | 2.00               | 4.04                    | 2.13                        | 106.55     | 6.55   |
| 20                   | 2.50               | 4.59                    | 2.68                        | 107.24     | 7.24   |
| 20                   | 3.00               | 5.03                    | 3.12                        | 103.95     | 3.95   |
| 20                   | 3.50               | 5.61                    | 3.70                        | 105.79     | 5.79   |
| 20                   | 4.00               | 6.13                    | 4.22                        | 105.46     | 5.46   |
| 20                   | 5.00               | 7.19                    | 5.28                        | 105.64     | 5.64   |
| 3                    | 6.00               | 8.12                    | 6.21                        | 103.45     | 3.45   |
| 20                   | 7.00               | 9.21                    | 7.30                        | 104.32     | 4.32   |
| 3                    | 8.00               | 10.08                   | 8.17                        | 102.17     | 2.17   |
| 20                   | 10.00              | 12.18                   | 10.27                       | 102.74     | 2.74   |
| 3                    | 12.00              | 13.97                   | 12.06                       | 100.48     | 0.48   |
| 20                   | 16.00              | >15.50                  | N/A                         | N/A        | N/A    |
| 16                   | 20.00              | 21.01                   | 19.10                       | 95.51      | 4.49   |
| 20                   | 32.00              | 26.55                   | 24.64                       | 77.02      | 22.98  |
| 3                    | 40.00              | 28.87                   | 26.96                       | 67.39      | 32.61  |

## APPENDIX 13

### Method Comparison Data

| Mean Measured<br>[Lactate] - X (ABL800) | Mean Measured<br>[Lactate] - X (Cobas400) | (Cobas400 +<br>ABL800) / 2 | Mean Difference<br>(ABL800-Cobas400) |
|-----------------------------------------|-------------------------------------------|----------------------------|--------------------------------------|
| 0.30                                    | 0.28                                      | 0.29                       | 0.02                                 |
| 0.54                                    | 0.57                                      | 0.56                       | -0.03                                |
| 1.08                                    | 1.09                                      | 1.08                       | -0.01                                |
| 1.53                                    | 1.61                                      | 1.57                       | -0.08                                |
| 2.02                                    | 2.13                                      | 2.08                       | -0.11                                |
| 2.56                                    | 2.68                                      | 2.62                       | -0.12                                |
| 2.97                                    | 3.12                                      | 3.04                       | -0.15                                |
| 3.44                                    | 3.70                                      | 3.57                       | -0.26                                |
| 3.91                                    | 4.22                                      | 4.06                       | -0.31                                |
| 4.92                                    | 5.28                                      | 5.10                       | -0.36                                |
| 5.48                                    | 6.21                                      | 5.84                       | -0.73                                |
| 6.56                                    | 7.30                                      | 6.93                       | -0.74                                |
| 7.15                                    | 8.17                                      | 7.66                       | -1.02                                |
| 9.16                                    | 10.27                                     | 9.72                       | -1.11                                |
| 10.38                                   | 12.06                                     | 11.22                      | -1.68                                |
| 17.58                                   | 19.10                                     | 18.34                      | -1.52                                |

## APPENDIX 14: Raw Data for Spiked Canine Plasma

ABL 800: Mean baseline lactate (x) = 1.82 mmol/L

| [Expected]<br>mmol/L | Measured Value (mmol/L) |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | Mean  |
|----------------------|-------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-------|
|                      | 1                       | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   |       |
| x + 0                | 1.8                     | 1.8  | 1.8  | 1.9  | 1.8  | 1.8  | 1.9  | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  | 1.8  | 1.9  | 1.9  | 1.8  | 1.8  | 1.82  |
| x + 0.25             | 2.0                     | 2.0  | 2.1  | 2.1  | 2.2  | 2.2  | 2.1  | 2.1  | 2.1  | 2.1  | 2.2  | 2.1  | 2.1  | 2.1  | 2.2  | 2.2  | 2.2  | 2.1  | 2.2  | 2.1  | 2.12  |
| x + 0.5              | 2.2                     | 2.4  | 2.3  | 2.3  | 2.3  | 2.5  | 2.3  | 2.3  | 2.4  | 2.4  | 2.4  | 2.3  | 2.4  | 2.4  | 2.4  | 2.4  | 2.4  | 2.4  | 2.4  | 2.4  | 3.65  |
| x + 1.0              | 2.8                     | 2.9  | 2.9  | 3.0  | 2.9  | 2.8  | 2.9  | 2.9  | 2.9  | 2.9  | 2.9  | 2.9  | 3.0  | 2.9  | 2.9  | 2.9  | 2.9  | 2.9  | 3.0  | 2.9  | 2.90  |
| x + 1.5              | 3.2                     | 3.4  | 3.4  | 3.4  | 3.4  | 3.4  | 3.3  | 3.3  | 3.4  | 3.4  | 3.3  | 3.3  | 3.3  | 3.4  | 3.4  | 3.4  | 3.4  | 3.3  | 3.3  | 3.3  | 3.35  |
| x + 2.0              | 3.8                     | 3.8  | 3.8  | 3.9  | 3.9  | 3.8  | 3.9  | 4.0  | 3.9  | 3.8  | 3.9  | 3.8  | 3.8  | 3.8  | 3.9  | 3.8  | 3.8  | 3.9  | 3.7  | 3.7  | 3.84  |
| x + 2.5              | 4.2                     | 4.3  | 4.4  | 4.4  | 4.4  | 4.4  | 4.5  | 4.3  | 4.3  | 4.4  | 4.4  | 4.4  | 4.3  | 4.5  | 4.5  | 4.5  | 4.3  | 4.3  | 4.4  | 4.3  | 4.38  |
| x + 3.0              | 4.8                     | 4.8  | 4.8  | 4.7  | 4.9  | 4.7  | 4.8  | 4.7  | 4.7  | 4.8  | 4.8  | 4.9  | 4.8  | 4.8  | 4.8  | 4.7  | 4.8  | 4.8  | 4.9  | 4.7  | 4.78  |
| x + 3.5              | 5.0                     | 5.2  | 5.3  | 5.3  | 5.3  | 5.3  | 5.2  | 5.2  | 5.3  | 5.2  | 5.2  | 5.3  | 5.4  | 5.3  | 5.3  | 5.3  | 5.3  | 5.4  | 5.3  | 5.1  | 5.26  |
| x + 4.0              | 5.5                     | 5.7  | 5.6  | 5.5  | 5.8  | 5.8  | 5.7  | 5.9  | 5.8  | 5.8  | 6.0  | 5.8  | 5.7  | 5.7  | 5.6  | 5.9  | *    | *    | *    | *    | 5.73  |
| x + 5.0              | 6.4                     | 6.6  | 6.6  | 6.5  | 6.6  | 6.8  | 6.6  | 6.7  | 6.8  | 6.7  | 6.7  | 6.9  | 6.8  | 6.8  | 6.8  | 6.9  | 7.0  | 6.9  | 7.0  | 6.7  | 6.74  |
| x + 6.0              | 7.2                     | 7.4  | 7.3  |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 7.3   |
| x + 7.0              | 8.1                     | 8.0  | 8.3  | 8.4  | 8.2  | 8.4  | 8.2  | 8.4  | 8.5  | 8.5  | 8.4  | 8.5  | 8.7  | 8.5  | 8.4  | 8.4  | 8.3  | 8.4  | 8.5  | 8.4  | 8.38  |
| x + 8.0              | 8.8                     | 9.0  | 9.1  |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 8.97  |
| x + 10.0             | 10.5                    | 10.7 | 10.9 | 10.8 | 10.8 | 11.3 | 10.9 | 11.2 | 10.9 | 10.9 | 11.5 | 10.9 | 11.1 | 11.4 | 11.1 | 11.3 | 10.8 | 11.1 | 10.8 | 10.8 | 10.98 |
| x + 12.0             | 12.0                    | 12.5 | 12.1 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 12.2  |
| x + 14.0             |                         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |       |
| x + 16.0             | 16                      | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16   | 16    |
| x + 20.0             | 18                      | 19   | 19   | 19   | 19   | 19   | 19   | 19   | 19   | 20   | 20   | 19   | 20   | 20   | 19   | 20   | 20   | 20   | 20   | 20   | 19.4  |
| x + 24.0             |                         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |       |
| x + 28.0             |                         |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |       |
| x + 32.0             | 27                      | 28   | 29   | 29   | 29   | 29   | 29   | 29   | 29   | 29   | 29   | 29   | 29   | 29   | 29   | 30   | 30   | 29   | 28   | 29   | 29.9  |
| x + 40.0             | High                    | High | High |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | N/A   |

\* Sample error

## APPENDIX 15: Raw Data for Spiked Canine Plasma

Coba400: Mean baseline lactate ( $\bar{x}$ ) = 1.91 mmol/L

| [Expected]<br>(mmol/L) | Measured Value (mmol/L) |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        | Mean  |
|------------------------|-------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|
|                        | 1                       | 2      | 3      | 4      | 5      | 6      | 7      | 8      | 9      | 10     | 11     | 12     | 13     | 14     | 15     | 16     | 17     | 18     | 19     | 20     |       |
| x + 0                  | 1.93                    | 1.92   | 1.91   | 1.91   | 1.91   | 1.91   | 1.90   | 1.91   | 1.90   | 1.91   | 1.91   | 1.90   | 1.90   | 1.91   | 1.90   | 1.92   | 1.91   | 1.91   | 1.91   | 1.91   | 1.91  |
| x + 0.25               | 2.20                    | 2.18   | 2.19   | 2.19   | 2.19   | 2.19   | 2.19   | 2.19   | 2.19   | 2.18   | 2.19   | 2.19   | 2.21   | 2.21   | 2.21   | 2.20   | 2.20   | 2.19   | 2.19   | 2.20   | 2.19  |
| x + 0.5                | 2.49                    | 2.48   | 2.47   | 2.47   | 2.47   | 2.51   | 2.48   | 2.46   | 2.48   | 2.48   | 2.48   | 2.47   | 2.48   | 2.48   | 2.48   | 2.49   | 2.46   | 2.47   | 2.47   | 2.47   | 2.48  |
| x + 1.0                | 3.01                    | 3.01   | 3.01   | 2.98   | 2.99   | 2.99   | 2.99   | 3.00   | 2.99   | 2.98   | 3.00   | 3.00   | 3.00   | 2.99   | 2.99   | 3.01   | 3.01   | 2.98   | 3.01   | 2.99   | 3.00  |
| x + 1.5                | 3.54                    | 3.52   | 3.52   | 3.52   | 3.53   | 3.53   | 3.53   | 3.52   | 3.52   | 3.51   | 3.52   | 3.55   | 3.55   | 3.51   | 3.51   | 3.51   | 3.51   | 3.51   | 3.50   | 3.50   | 3.52  |
| x + 2.0                | 4.01                    | 4.03   | 4.03   | 4.03   | 4.02   | 4.05   | 4.03   | 4.07   | 4.06   | 4.03   | 4.03   | 4.05   | 4.04   | 4.03   | 4.06   | 4.05   | 4.03   | 4.04   | 4.06   | 4.06   | 4.04  |
| x + 2.5                | 4.59                    | 4.59   | 4.59   | 4.63   | 4.59   | 4.59   | 4.6    | 4.59   | 4.59   | 4.60   | 4.58   | 4.60   | 4.60   | 4.59   | 4.63   | 4.59   | 4.60   | 4.54   | 4.57   | 4.55   | 4.59  |
| x + 3.0                | 5.05                    | 5.04   | 5.01   | 5.00   | 5.04   | 5.05   | 5.01   | 5.03   | 5.05   | 5.03   | 5.02   | 5.01   | 5.04   | 5.02   | 5.04   | 5.02   | 5.02   | 5.04   | 5.02   | 5.02   | 5.03  |
| x + 3.5                | 5.62                    | 5.61   | 5.61   | 5.60   | 5.60   | 5.63   | 5.67   | 5.63   | 5.59   | 5.62   | 5.60   | 5.61   | 5.58   | 5.63   | 5.61   | 5.61   | 5.60   | 5.58   | 5.60   | 5.64   | 5.61  |
| x + 4.0                | 6.10                    | 6.14   | 6.10   | 6.10   | 6.12   | 6.14   | 6.14   | 6.13   | 6.12   | 6.14   | 6.13   | 6.11   | 6.12   | 6.15   | 6.11   | 6.11   | 6.15   | 6.15   | 6.18   | 6.12   | 6.13  |
| x + 5.0                | 7.21                    | 7.15   | 7.21   | 7.16   | 7.16   | 7.19   | 7.21   | 7.22   | 7.16   | 7.22   | 7.23   | 7.19   | 7.19   | 7.20   | 7.20   | 7.16   | 7.19   | 7.19   | 7.15   | 7.24   | 7.19  |
| x + 6.0                | 8.16                    | 8.09   | 8.10   |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        | 8.12  |
| x + 7.0                | 9.33                    | 9.25   | 9.28   | 9.22   | 9.30   | 9.22   | 9.23   | 9.20   | 9.25   | 9.18   | 9.23   | 9.13   | 9.19   | 9.23   | 9.18   | 9.13   | 9.19   | 9.16   | 9.14   | 9.20   | 9.21  |
| x + 8.0                | 10.12                   | 10.06  | 10.07  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        | 10.08 |
| x + 10.0               | 12.21                   | 12.23  | 12.18  | 12.22  | 12.14  | 12.21  | 12.17  | 12.07  | 12.21  | 12.20  | 12.18  | 12.19  | 12.21  | 12.16  | 12.23  | 12.14  | 12.19  | 12.15  | 12.20  | 12.18  | 12.18 |
| x + 12.0               | 14.07                   | 13.90  | 13.93  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        | 13.97 |
| x + 14.0               |                         |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |       |
| x + 16.0               | >15.50                  | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | >15.50 | N/A   |
| x + 20.0               | 21.11                   | 21.23  | 21.25  | 21.14  | 20.92  | 21.03  | 21.02  | 21.05  | >15.50 | >15.50 | 20.77  | 20.96  | 20.78  | 20.92  | 20.85  | 21.10  | >15.50 | >15.50 | 20.93  | 21.01  | 21.01 |
| x + 24.0               |                         |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |       |
| x + 28.0               |                         |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |       |
| x + 32.0               | 26.44                   | 26.27  | 26.26  | 26.91  | 26.68  | 26.97  | 26.80  | 26.68  | 27.52  | 27.07  | 27.04  | 26.72  | 26.90  | 26.79  | 26.81  | 26.65  | 26.25  | 25.83  | 25.72  | 24.78  | 26.55 |
| x + 40.0               | 28.41                   | 29.09  | 29.10  |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        | 28.87 |