

Sources of error in acid-base analysis from a blood gas analyser result: a narrative review

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Preservation of blood pH within a narrow range is essential to optimal physiological function. This narrow pH range is maintained via the interactions of various buffer systems. Blood gas analysis is thus essential in the diagnosis and management of disorders affecting blood pH. Common methods of acid-base interpretation in veterinary science are the traditional approach, the physicochemical approach and the semiquantitative approach. However, blood gas analysis is prone to error during the pre-analytical, analytical and post-analytical phases of the laboratory process. The pre-analytical phase incorporates steps in obtaining the sample, thus sources of pre-analytical error are related to operator technique. Most errors occur during the pre-analytical phase. Pre-analytical errors include entrainment of air bubbles into the sample and delays between sampling and analysis, both of which cause inaccurate measurement of oxygen and carbon dioxide tensions. The analytical phase outlines processes within the analyser. Common analytical errors are related to substances confounding analyte measurements. The post-analytical phase mainly describes interpretation of the results. Some of the approaches to acid-base interpretation require extensive post-analytical calculations, thus lending themselves to error. Errors occurring during the prior phases will be amplified. Errors in the measurement of the carbon dioxide tension (from which bicarbonate concentration and base excess are calculated) will introduce error into all three methods of acid-base interpretation. Furthermore, errors occurring in the measurements of electrolytes and lactate will result in incorrect interpretations if the physicochemical and semiquantitative approaches are applied. The potential sources of error during the various phases are reviewed.

Keywords: pre-analytical phase, analytical phase, post-analytical phase, analytical errors, blood pH, Henderson-Hasselbalch, physicochemical approach, semiquantitative approach

Introduction

The body strives to maintain blood pH within a narrow range in order to optimise functioning of the cardiovascular and nervous systems as well as miscellaneous metabolic functions (Kuleš et al. 2015; Monnig 2013). The resultant pH is due to a complex balance between various intra- and extracellular buffer systems, plasma ion concentrations and functional interaction of the respiratory and renal systems (Hopper et al. 2014a; Hopper et al. 2014b; Monnig 2013). Thus, blood gas testing for acid-base analysis has become an indispensable tool in the management and prognostication of critically ill patients (Hopper et al. 2014a).

Requesting laboratory tests has been described as a brain-to-brain loop, commencing with the conception of requesting the test and culminating in the interpretation thereof and the resulting therapeutic interventions (Lundberg 1981). Furthermore, the laboratory process has been traditionally divided into three phases (Baird 2013). Firstly, the pre-analytical phase, which includes steps which occur prior to introducing a sample into an analyser. Secondly, the analytical phase which describes the processes within the analyser itself. Lastly, the post-analytical phase relates to steps occurring after obtaining the test result, including data management, calculation of certain variables from variables measured by the analyser and interpretation of the result (Baird 2013). Errors occurring during the laboratory process are not exclusive to blood gas testing for acid-base analysis. However, the physicochemical properties of

blood gas analytes make them more prone to these errors (Baird 2013).

Clinicians making use of blood gas testing for acid-base analysis should therefore be aware of the potential errors during the laboratory process, including methods of recognising and mitigating these errors. The aim of this review is to highlight the pre-analytical, analytical and post-analytical errors that are frequently encountered when analysing acid-base status using a blood gas test result.

The data sources underpinning this review comprise original research articles, review articles, case reports, textbook chapters and clinical reference websites. These sources were found online by typing the following terms into a search engine (<http://www.google.com>): "blood gas errors", "pre-analytical blood gas errors", "analytical blood gas errors", "post-analytical blood gas errors". These search terms were also amended to include "veterinary", "dog", "canine", "cat", "feline", "horse", "equine", and "ruminant" in order to gain more relevant results. A search engine was used instead of traditional research databases (PubMed, Web of Science and CAB abstracts) for its ease of use as well as the wider variety of results obtained. The first page of the results was examined for journal articles and well-referenced anaesthesia and critical care resources, with a focus on veterinary-related sources. The first page of results was used only, as the relevancy of the results decreased, from the first result onward. A total of 95 discrete results were obtained using this search strategy.

Of these 95 results, 59 were used to develop the manuscript outline. Reasons for rejecting the remainder of the results were: articles not in the English language, unreferenced clinical guides and blogs, user manuals for blood gas machines and resources that were irrelevant to the subject of the manuscript. Further sources were obtained by perusing the reference lists of sources obtained using the above strategy.

Pre-analytical errors

The pre-analytical phase is arguably the most sensitive to errors. Up to 62% of errors during the laboratory process occur during this phase in human clinical pathology laboratories (Hawkins 2012).

Patient factors

Before ordering a laboratory test, it is prudent to decide whether the analysis is indicated. Discussing the indications for blood gas analysis is beyond the scope of this clinical review, however, an anaerobic arterial blood sample is considered the gold standard to assess both respiratory function and acid-base status (McCanny et al. 2012; Williams et al. 2014). Considering the technical difficulty and risk of iatrogenic complications associated with obtaining an arterial blood sample (Malatesha et al. 2007; McCanny et al. 2012; Williams et al. 2014), a venous blood sample may be sufficient to assess the carbon dioxide tension (PCO_2) as well as the metabolic contributors to blood pH (Ilkiw et al. 1991; Malatesha et al. 2007; McCanny et al. 2012; Tamura et al. 2015). In this light, Ilkiw et al. (1991) and Tamura et al. (2015) compared arterial and venous blood gas analytes in conscious dogs and found good correlation between the pH, bicarbonate (HCO_3^-) and base excess (BE). Similarly, Kadwa et al. (2022) found good agreement between arterial and venous blood pH, HCO_3^- and BE, but not PCO_2 , in anaesthetised dogs with respiratory acidosis. Furthermore, it must be borne in mind that low-flow states (severe hypovolaemic shock with impending circulatory collapse), will significantly increase the arteriovenous PCO_2 gradient, potentially resulting in a large disparity between the arterial and venous blood pH. Therefore, it is recommended that in haemodynamically unstable patients, an arterial blood sample be used to assess respiratory function and acid-base status. The arteriovenous PCO_2 difference has been investigated as marker of global tissue perfusion in humans suffering septic shock and a rabbit model of haemorrhagic shock, where a difference of > 6 mmHg may be an indicator of insufficient perfusion (Vallée et al. 2008; Williams et al. 2014). It has also been investigated as marker of adequacy of cardiopulmonary resuscitation in dogs (Sato et al. 1994). Similarly, Zeiler et al. (2022), in a feline acute haemorrhage-resuscitation model, found a significant increase in the venous PCO_2 in cats that underwent acute haemorrhage versus the control group. Thus, arterial and venous samples (specifically the PCO_2) can be compared to assess the adequacy of resuscitative effects in shock states, however, the utility of the arteriovenous PCO_2 gradient has not been completely validated in dogs.

Test over-utilisation is a common occurrence in resource-rich settings (Baird 2013), with serious consequences for patients. Lynch et al. (2016) found a moderate correlation between the

number of phlebotomies performed and hospital-acquired anaemia (packed cell volume < 0.35 L/L in dogs and < 0.3 L/L in cats) in critically ill cats and dogs. When indexed to the duration of hospitalisation, patients that did not develop anaemia had a median of 1.25 phlebotomies performed per day, whereas patients that developed anaemia had a median of 1.67 phlebotomies performed per day. Additionally, patients that required blood products had a median of two phlebotomies performed per day. Furthermore, Lynch et al. (2016), also concluded that cats were more likely than dogs to develop hospital-acquired anaemia and that surgical patients were at increased risk of developing hospital-acquired anaemia as compared to medical patients. Furthermore, repeated blood gas analysis can account for up to 40% of blood loss in critically ill human patients (Andrews & Waterman 2008). It is therefore recommended that hospitalised cats and dogs do not undergo more than one phlebotomy per day, especially in cats and surgical patients, unless the results of the tests are essential to lifesaving therapeutic interventions.

Sampling and storage factors

The determination of oxygen (PO_2) and carbon dioxide tensions in blood are amongst the major indications for blood gas analysis (Irizarry & Reiss 2009), with PCO_2 being the only gaseous variable contributing to acid-base physiology and interpretation (Monnig 2013). The tendency of these gases within the blood to rapidly equilibrate with the tensions of their ambient counterparts gives rise to unique considerations during collection of a sample for blood gas analysis (Baird 2013; Magee 2012). Gas tight syringes are required to prevent equilibration with the environment (Baird 2013). Historically, glass syringes were used for this purpose, however, safety concerns have led to the adoption of plastic syringes (Baird 2013). It is imperative that any air bubbles entrained during the collection process be rapidly expelled as equilibration between the blood and air bubbles occurs within two minutes (Biswas et al. 1982). Typical results of samples exposed to air would be a tendency for the PO_2 to approach ambient levels with a reduction in the PCO_2 . Consequently, less carbonic acid will be produced, leading to an increased pH, resulting in a greater binding of ionised calcium to albumin. A false hypocalcaemia may ensue (Baird 2013). Prior guidelines recommended the storage of samples on ice to prevent metabolic consumption of oxygen, however, low temperatures are hypothesised to cause contracture of the polymers in plastic syringes, making them permeable to oxygen, but not carbon dioxide (Baird 2013). Picandet et al. (2007) reported the effects of storage temperature and time to analysis on arterial blood of horses collected into plastic syringes. They concluded that the PO_2 of a sample stored in iced water ($0-5^\circ\text{C}$) significantly increased (> 10 mmHg) within five minutes, compared to 20 minutes for a sample stored at ambient temperature ($22-25^\circ\text{C}$). Furthermore, they also found that PCO_2 remained stable for 83 minutes when the sample was stored in a plastic syringe at ambient temperature. Therefore, a blood gas sample withdrawn into a plastic syringe should be stored at room temperature and analysis ideally performed within 10–15 minutes, if the PO_2 is required (Baird 2013; Biswas et al. 1982).

Modern blood gas syringes are preloaded with lyophilised, cation-balanced heparin as an anticoagulant. Conversely, liquid heparin is a poly-anion which can bind cations within the sample. Additionally, excess liquid heparin (> 10% of the sample volume) causes dilution of analytes as well as equilibration of the partial pressures of gases within the heparin and the sample (Baird 2013; Hutchison 1983; Monnig 2013). Another important cause of analyte dilution and sample contamination is the aspiration of a sample from an indwelling vascular catheter with fluid within the line (Baird 2013). In order to limit dilution of a sample aspirated from an indwelling vascular catheter, the prior recommendation was to aspirate a waste sample (pre-sample) representing 300% of the catheter dead space, aspirate the actual sample and reinfuse the pre-sample in order to limit iatrogenic anaemia. Problems with reinfusion of the pre-sample include: infusion of blood clots, haemolysis, haemodilution, contamination of the pre-sample and accidental submission of the pre-sample (Barr et al. 2017). The push-pull technique was devised to minimise these risks. The pre-sample (300% catheter dead space volume) is aspirated and reinjected three times without disconnecting the syringe. A second syringe is then used to obtain the sample. Barr et al. (2017) compared this technique, to direct venipuncture, on venous blood gas analysis in dogs, before and after anaesthesia for elective surgeries. Samples drawn from a 20G catheter placed in a cephalic vein were compared to direct aspiration from the contralateral cephalic vein. There were statistically significant differences between the collection techniques for PCO_2 , BE and HCO_3^- , chloride, ionised calcium and potassium concentrations. However, these differences were deemed clinically irrelevant on the basis that all the values remained within their respective reference ranges for the laboratory used in the study. Furthermore, a high level of agreement between the techniques was found using Bland-Altman plots; the biases calculated from the Bland-Altman plots were small and were within the allowable limits of analytic error for clinical laboratory tests established by the American Society of Veterinary Clinical Pathologists. The push-pull technique may therefore be suitable for sampling of canine blood for blood gas analysis via an over-the-needle catheter. However, further investigation is required to determine the suitability of this technique for blood gas analysis on samples aspirated from over-the-wire catheters.

Fluctuations in the arterial PO_2 during the respiratory cycle have been described in dogs, cats and lambs (Bergman 1961; Folgering et al. 1978; Purves 1966). These fluctuations are clinically irrelevant, and thus, no difference in arterial PO_2 values will be observed in samples obtained at varying points in the respiratory cycle (Pfeiffer et al. 2006). However, large fluctuations of 439 mmHg (Baumgardner et al. 2002) and 283 mmHg (Pfeiffer et al. 2006) in arterial PO_2 were noted between inspiration and expiration in surfactant-depleted models of lung injury in mechanically ventilated rabbits. The PO_2 in these studies was continuously measured via a probe placed in the brachiocephalic artery (Baumgardner et al. 2002; Pfeiffer et al. 2006). It is theorised that the reason for these fluctuations is tidal recruitment (repetitive opening and closing of collapsed alveoli during the mechanical respiratory cycle [Tusman et al. 2015]) during inspiration (Baumgardner et al. 2002; Pfeiffer et

al. 2006). Pfeiffer et al. (2006) recommend that arterial blood gas samples used to assess average oxygen delivery should be drawn over two to three respiratory cycles to obtain the mean arterial PO_2 . Ideally, this recommendation should also be applied in veterinary species, especially in patients suffering pulmonary diseases. However, this recommendation may only be practical in anaesthetised patients, considering the potentially fractious reaction to arterial blood sampling in veterinary species. Furthermore, it may not be possible to collect a sample over three respiratory cycles in species with low respiratory rates (horses, for example) without the sample clotting.

Haemolysis is another common cause of introduction of pre-analytical error into the laboratory process. Massive haemolysis in human blood samples can cause false elevations of intra-erythrocytic electrolytes (mainly potassium) and dilution of other electrolytes (mainly sodium and calcium), causing falsely low values (Buckley & Russel 1988; Delgado et al. 2019; Dukić et al. 2016; Lippi et al. 2013). This error is due to the high intra-erythrocytic potassium concentration of human cells (Dugdale 2010; Leard et al. 1990), however, a false elevation in potassium does not occur in haemolysed samples of canine blood due to the significantly lower intra-erythrocytic potassium concentrations of dog erythrocytes as compared to human erythrocytes (10 mEq/L versus 155 mEq/L) (Bernadini et al. 2009; Leard et al. 1990). Nonetheless, false elevations in potassium (up to 10.9 mEq/L, reference range 3.6–6) have been described in haemolysed blood samples from Japanese dog breeds (Akita, Shiba, Jindo) and their crosses (Battison 2007). This phenomenon is thought to be as a result of a 10- to 13-fold higher concentration of intra-erythrocytic potassium concentrations in Japanese breeds as compared to other breeds (Battison 2007). Similar deviations are likely to occur with haemolysis of blood from horses, pigs and Holstein cattle, also due to their high intra-erythrocytic potassium concentrations (Battison 2007). Ionised calcium concentrations in human blood samples have been found to decline between 7% and 40% from the true values, depending on the degree of haemolysis (Buckley & Russel 1988; Lippi et al. 2013). Despite multiple studies investigating the effects of haemolysis on many analytes (Lippi et al. 2006a, Lippi et al. 2011), its effects on sodium concentrations has been poorly described (Delgado et al. 2019). Various correction formulae for potassium (Dimeski et al. 2005) and sodium concentrations (Delgado et al. 2019) have been described using free plasma haemoglobin as a correction factor for haemolysis. These formulae have not been described in veterinary species. Thus, the most practical solution in the face of a result that does not agree with the clinical picture would be to repeat the test, paying attention to sources of error if no other pathophysiological explanations are viable.

The major causes of haemolysis are thought to be aspiration of samples via narrow gauge needles and plastic intravenous catheters (Burns & Yoshikawa 2002; Lippi et al. 2006b; Sharp & Mohammad 2003). However, Sharp & Mohammad (2003) demonstrated that 18G needles produced the most and 22G needles the least haemolysis during phlebotomy. Unfortunately, Sharp and Mohammad (2003) could not provide a concrete reason for these counterintuitive results; they theorised that the samples drawn via the 18G needles may have been exposed to

unknown shear stresses elsewhere in the phlebotomy apparatus. In another study, Lippi et al. (2006b) compared the influence of haemolysis on electrolytes in samples aspirated via 21G, 23G and 25G needles. They found that samples aspirated via 21G and 23G did not introduce sufficient haemolysis into the samples to interfere with sample analysis, however, samples aspirated via a 25G needle were sufficiently haemolysed to introduce an unacceptable margin of error into the analysis of calcium, chloride, potassium and sodium. Furthermore, Burns and Yoshikawa (2002) concluded that sample aspiration through a 20G plastic intravenous catheter reduces the risk for haemolysis when compared to a 22G catheter. Similarly, Kennedy et al. (1996) found a 100% haemolysis rate in samples aspirated via a 24G catheter, a 15% haemolysis rate in samples collected via a 20G catheter and a 0% haemolysis rate in samples collected via 18G and 16G catheters. In addition, excessive aspiration force during aspiration and residual alcohol-based disinfectants on the skin have been reported to cause haemolysis (Carraro et al. 2000). The World Health Organization (WHO) guidelines on drawing blood (2010) recommend thorough drying of a blood collection site after cleaning with an alcohol-based disinfectant. However, Salvango et al. (2013) and Sarmah et al. (2016) disputed the evidence behind this recommendation. In both studies, sampling was performed after skin preparation according to the WHO guidelines, these were compared to paired samples collected without drying the alcohol-based disinfectant used to clean the skin. Both Salvango et al. (2013) and Sarmah et al. (2016) concluded that not drying the skin of an alcohol-based disinfectant before sample collection does not lead to the development of haemolysis in the sample collected. Therefore, it is recommended that samples for blood gas analysis be drawn using a 22G or 23G needle or aspirated via a catheter 20G in size or larger. Excessive force should not be applied during aspiration. Figure 1 presents a suggested sequence of events for obtaining a sample for blood gas analysis.

Transport factors

Pneumatic vacuum tube systems are commonly used to transport blood samples from patient side to the laboratory in large hospital settings. These have the potential to introduce pre-analytical error by exposure of up to 15 times gravitational acceleration (147 m/s^2), resulting in vigorous agitation of the sample leading to quicker gas equilibration from small air bubbles within the sample (Baird 2013). Victor Peter et al. (2011)

reported deviations (increases and decreases) in arterial PO_2 of up to 40 mmHg when comparing paired blood gas samples transported to a laboratory by hand or a pneumatic vacuum system. Additionally, pneumatic vacuum systems have also been implicated in haemolysing samples when the acceleration within the system exceeds twice gravitational acceleration and the speed within the systems exceeds 2 m/s (Burns & Yoshikawa 2002; Mullins et al. 2016; Tiwari et al. 2012). It is thus recommended that if erroneous blood gas results are suspected, the pneumatic vacuum tube system be investigated as a potential cause. Furthermore, acceleration and speed must be considered during upgrading of existing systems or construction of new systems.

Analytical errors

The modern blood gas analyser aspirates a presented sample into a temperature-controlled test chamber stabilised at 37°C . The sample is then sequentially passed through a series of electrodes which directly measure the pH, PO_2 and PCO_2 , electrolyte and lactate concentrations (Irizarry & Reiss 2009; Magee 2012). The HCO_3^- , BE and anion gap (AG) are derived from the measured variables by means of pre-programmed formulae and nomograms, as presented in Table 1 (Irizarry & Reiss 2009; Magee 2012; Siggaard-Andersen 1977). Thus, pre-analytical

Table 1: Source of the variables displayed on the printout from a typical blood gas analyser

Variable	Derivation
pH	Directly measured
PCO_2	Directly measured
PO_2	Directly measured
Oxygen saturation	Derived from the haemoglobin-oxygen dissociation curve
HCO_3^-	Calculated from the Henderson-Hasselbalch Equation
BE	$\text{HCO}_3^- - 24.4 = -(2.3 \times \text{Hb} + 7.7) \times (\text{pH}^\circ - 7.40) + \text{BE} / (1 - 0.023 \times \text{Hb})$ (Van Slyke Equation)
Electrolytes (Na^+ , K^+ , Ca^{++} , Cl^-)	Directly measured
Anion gap	$(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)$
Lactate	Directly measured

PCO_2 – carbon dioxide tension, PO_2 – oxygen tension, HCO_3^- – bicarbonate concentration, BE – base excess, Hb – haemoglobin concentration, pH° – pH of plasma at 37°C , Na^+ – sodium, K^+ – potassium, Ca^{++} – calcium, Cl^- – chloride

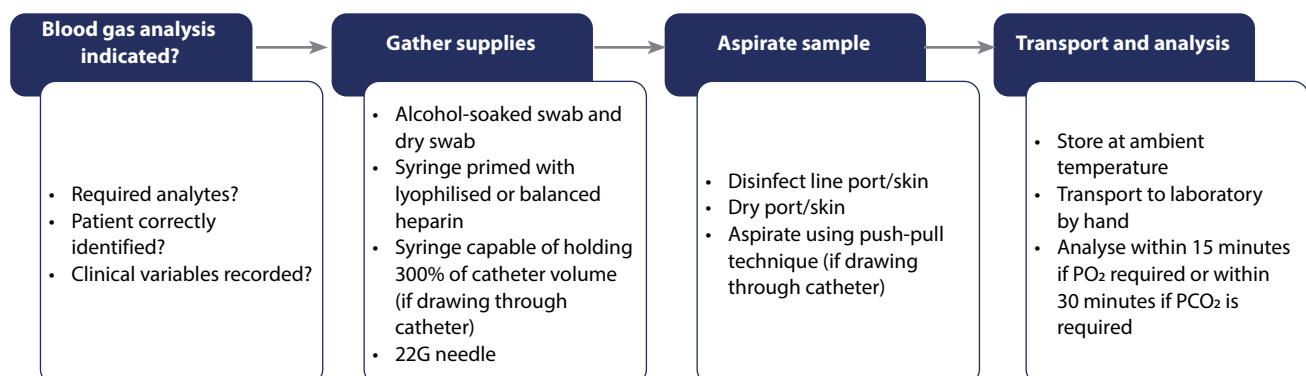


Figure 1: Suggested steps for obtaining and analysing a sample for acid-base analysis using blood gas analysis

errors affecting the measured variables will directly affect the derived variables.

Errors occurring during the analytical phase can arise from problems within the analyser itself (poor quality control methods) or substances within the blood sample confounding analysis (such as selectivity failure of ion selective electrodes with similar ions) (Peacock & Smart 2013).

Quality control (QC) is essential for blood gas analysers as these analyses are usually performed on critically ill patients (Dukić et al. 2016). A complete discussion on QC is beyond the scope of this review. Briefly, the performance of the analyser is measured against a known standard; thus, clinicians can trust the repeatability of results reported (Dukić et al. 2016). The various electrodes have a finite lifespan and lose their accuracy over time due to deposition of blood proteins (Magee 2012). Therefore, newer analysers employ either single-use cartridges or cartridges which require replacement after 28–30 days (manufacturer dependant) (Radiometer n.d.; Abbott Point of Care n.d.; Siemens Healthineers n.d.). Moreover, these cartridges have automated QC capability which requires minimal operator input (Dukić et al. 2016).

Ionised electrolytes with similar ionisation could compete at the ion selective electrode when measuring the standard electrolytes used in acid-base analysis (Peacock & Smart 2013). These standard electrolyte ions are sodium (Na^+), potassium (K^+), calcium (Ca^{++}) and chloride (Cl^-) (Peacock & Smart 2013). An example of similar ions competing is that of elevated bromide concentration, which has been reported as a cause of a false hyperchloraemia in a dog which consumed potassium bromide supplemented horse feed (Peacock & Smart 2013). This phenomenon may also have theoretical implications in patients where potassium bromide is used as an anti-epileptic, especially if a gross overdose has occurred. Additionally, elevated plasma salicylate (aspirin) concentrations, especially in cases of acute intoxication, may also result in a spurious hyperchloraemia (Kashani et al. 2018). In addition to bromides and salicylates, thiocyanates (a product of cyanide metabolism) and iodides may also produce a false hyperchloraemia (Dukić et al. 2016).

In cases of ethylene glycol intoxication, a blood gas analyser may report a falsely elevated lactate level. In analysers where lactate concentration is measured by amperometry, the lactate within the sample is converted to pyruvate and hydrogen peroxide by lactate oxidase. The subsequent reduction of hydrogen peroxide produces an electrical current in proportion to the sample lactate concentration (Marwick et al. 2012). Glycolate, a product of ethylene glycol metabolism, is also a substrate for lactate oxidase, giving rise to a spuriously elevated lactate in cases of antifreeze toxicity (Marwick et al. 2012). Spectrophotometric assays using lactate dehydrogenase are not prone to this analytical error. The disparity between reported lactate levels utilising the different assay methods is known as the 'lactate gap', a hallmark of ethylene glycol intoxication (Marwick et al. 2012). Lactate naturally exists in two isoforms: L-lactate and D-lactate. The L-isoform is the most abundant in mammalian blood, existing at 50 to 200 times the level of the D-isoform during homeostasis (Higgins 2011). The L-isoform predominates during pathophysiological states

characterised by hypoperfusion and tissue hypoxia (type A hyperlactataemia). However, elevated L-lactate concentrations could also arise as a result of underlying disease resulting in decreased clearance, toxins and drugs interfering with oxidative phosphorylation or inborn errors of mitochondrial metabolism (type B1, B2 and B3, respectively) (Dugdale 2010; Higgins 2011; Pang & Boysen 2007). Neonatal diarrhoea in calves, D-lactic acid neurotoxicity in lambs (drunken lamb syndrome, a condition hypothesised to occur secondary to excessive ruminal or colonic fermentation) and short bowel syndrome in human beings are the only reported conditions to elevate D-lactate to clinically significant concentrations ($> 3 \text{ mmol/L}$) (Angell et al. 2013; Lorenz et al. 2005). Both the lactate oxidase and dehydrogenase assays are specific for L-lactate; thus, a D-lactate-specific assay must be employed in cases where acidosis due to elevated D-lactate is suspected (Higgins 2011). Concerns also arise with infusions of Lactated Ringers' Solution (LRS), which usually contains a racemic mixture of lactate. However, an infusion thereof has been shown not to elevate D-lactate to clinically significant concentrations in human patients (Higgins 2011). Similarly, in healthy dogs, infusions of lactate containing fluids at clinically relevant rates, 10 ml/kg/hour for two hours or 30 ml/kg/hour for one hour had no effect on blood lactate concentrations (Muir 2017). However, a large volume of LRS (180 ml/kg over an hour) in healthy dogs was shown to significantly elevate blood lactate concentrations, likely due to lactate concentrations in excess of the metabolic capacity (Muir 2017). Furthermore, blood samples obtained from indwelling catheters improperly cleared of intravenous fluids will affect blood lactate results. Fluids which contaminate the catheter and do not contain lactate, will falsely decrease blood lactate concentrations through a dilutional effect, whereas fluids containing lactate will falsely elevate lactate concentrations (Pang & Boysen 2007). Moreover, patient restraint and struggling, especially in cats, are known to elevate blood lactate. Stress hyperglycaemia has been shown to be associated with hyperlactataemia due to struggling (Allen & Holm 2008). Thus, Allen and Holm (2008) have recommended obtaining blood glucose concentrations simultaneously with blood lactate concentrations to differentiate stress from underlying disease.

Anaesthetic and analgesic drugs inhibit thermoregulation in a dose-dependant manner which results in an increase in the body temperature interthreshold range from 0.2 °C to 4 °C (Sessler 1997). Thus, a controversial topic in both human and veterinary medicine arises – temperature correction of the blood gas results to the patient's temperature during analysis (Figure 2) (Abdul Aziz & Meduoye 2010; Brandis 2015). However, temperature correction only becomes relevant during significant deviations from physiological temperatures ($> 2^\circ\text{C}$ outside the physiological range), typically, deep hypothermic cardiopulmonary bypass (Abdul Aziz & Meduoye 2010; Brandis 2015). With a decrease in temperature, solubility of gases in blood as well as the dissociation constant of water (thus the pH of neutrality) increases (Brandis 2015; Wang & Jackson 2016). Intracellular pH is maintained at the pH of neutrality with a change in temperature via the buffering capacity of the intracellular proteins (Brandis 2015). Optimal intracellular functioning occurs at a $[\text{OH}^-]:[\text{H}^+]$

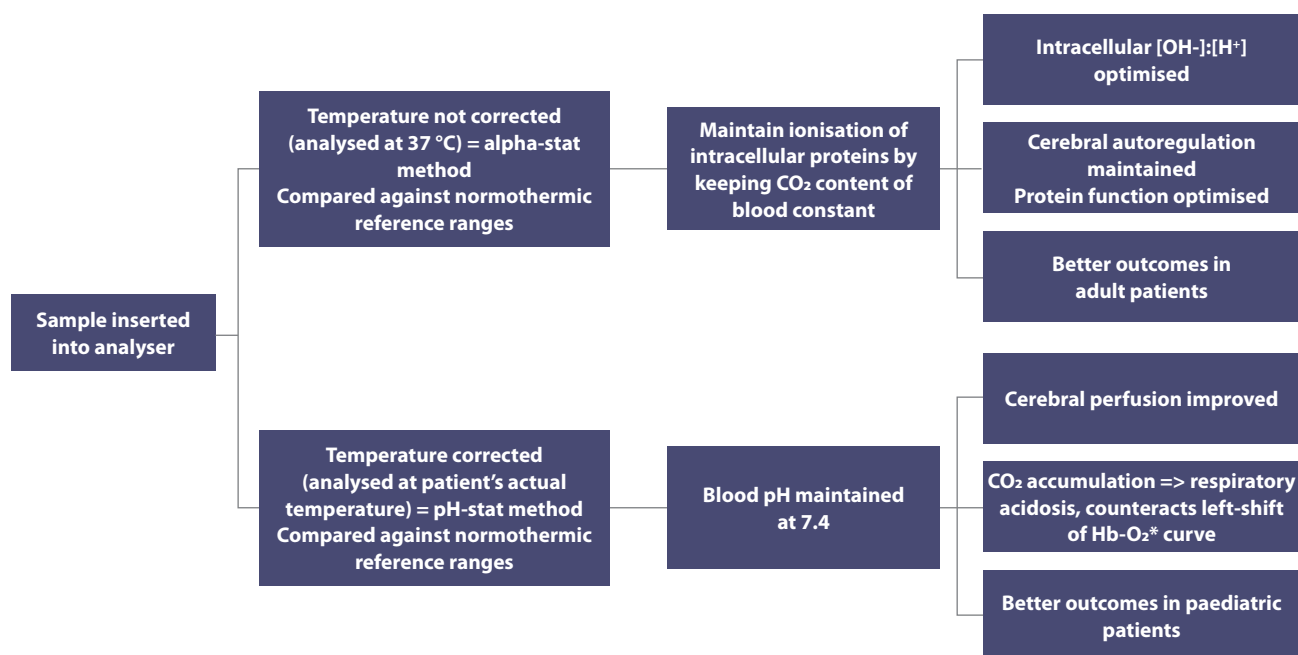


Figure 2: A summary of the alpha-stat versus pH-stat methods of blood gas analysis

*Hb-O₂ – Haemoglobin oxygen dissociation curve

ratio of 16:1 (Dugdale 2010; Muir 2015). The imidazole group of the histidine residues of these proteins facilitates this ratio by maintaining its degree of ionisation (alpha) at 0.55 over a range of temperatures (Brandis 2015; Wang & Jackson 2016). However, imidazole alpha will only remain constant if the carbon dioxide content of blood is kept constant over a range of temperatures (Brandis 2015; Wang & Jackson 2016). Thus, the alpha-stat theory aims to maintain the intracellular [OH⁻]:[H⁺] ratio by keeping the imidazole alpha constant (Brandis 2015; Wang & Jackson 2016). Blood gas samples are analysed at 37 °C and interventions are instituted based against normothermic reference ranges (Dugdale 2010). Alternatively, the pH-stat theory aims to maintain blood pH at 7.4 regardless of body temperature. Blood gas samples are analysed at the patient's actual temperature and interventions are instituted based against normothermic reference ranges (Dugdale 2010). With decreases in body temperature, solubility of carbon dioxide in blood increases. Thus, ventilatory management according to the pH-stat method allows a greater accumulation of carbon dioxide at lower temperatures and therefore a respiratory acidosis ensues (Brandis 2015). Proponents of the alpha-stat theory claim that cerebral autoregulation is better maintained, whereas proponents of the pH-stat theory claim that cerebral perfusion is improved (Abdul Aziz & Meduoye 2010). Regardless, human studies into cardiopulmonary bypass have found better outcomes with the alpha-stat method in adults and the pH-stat strategy in paediatric patients (Abdul Aziz & Meduoye 2010). However, the alpha-stat method seems to be more widely accepted in human medicine (Brandis 2015).

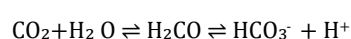
Post-analytical errors

The post-analytical phase is the culmination of the brain-to-brain loop which commenced with the pre-analytical phase of processing a blood gas sample. Errors during this phase of the laboratory process can stem from poor data management, analyser differences and amplification of errors accrued

during the pre-analytical and analytical phases (Lundberg 1981). Moreover, various schools of thought concerning the interpretation of acid-base status using blood gas tests can further augment these errors.

Ideally, clinicians should follow a hypothetico-deductive method in pursuing a diagnosis. However, clinicians may develop cognitive short-cutting strategies, especially in time- and resource-constrained environments (Croskerry 2002). These strategies are formed by clinical experience, impressions of patients, pattern recognition and prevailing socio-economic circumstances (Croskerry 2002; Gopal et al. 2021). Therefore, unfortunately, these strategies are prone to a host of cognitive biases (Croskerry 2002; Gopal et al. 2021), the scope of which is beyond this review. Briefly, blood gas analysis, which can comprise 10 or more steps in its complete interpretation, can fall prey to these biases at every step, possibly resulting in incorrect diagnoses (Croskerry 2002). Ideally, clinicians should be aware of these biases and practise debiasing strategies in order to limit the incidence of medical error (Croskerry 2002). Briefly, the debiasing strategies recommended by Croskerry (2002) are to be aware of the typical pitfalls associated with a particular process in order to better avoid them, as well as critically evaluating one's own thought processes.

Currently, there exist several approaches to acid-base analysis and interpretation and three common veterinary methods are highlighted in Table II (Hopper et al. 2014a). The traditional approach aims to describe blood pH by its chemical relationship with HCO₃⁻ and PCO₂. Carbon dioxide formed during metabolic processes combines with water to form carbonic acid, which dissociates into HCO₃⁻ and hydrogen ions, as follows (Hopkins et al. 2020):



The traditional approach applies the Henderson-Hasselbalch equation to the above process to determine blood pH by its

Table II: Summary of the approaches to acid-base analysis

Method	Traditional approach (Henderson-Hasselbalch equation)	Physicochemical approach (Stewart method)	Semiquantitative approach (Fencel et al. 2000)
Variables of interest	PCO ₂ , HCO ₃ ⁻	PCO ₂ , A _{TOT} , SID	BE, effects of free water, chloride, albumin, phosphorous, lactate
Quantification of unmeasured anions	BE and AG	SIG	XA

PCO₂ – carbon dioxide tension, A_{TOT} – plasma weak acids, SID – strong ion difference, BE – base excess, AG – anion gap, SIG – strong ion gap, XA – unmeasured anions

metabolic (HCO₃⁻) and respiratory (PCO₂) contributors (Chawla & Drummond 2008; Kellum 2005; Monnig 2013):

$$\text{pH} = 6.1 + \log \frac{[\text{HCO}_3^-]}{(S \times \text{PCO}_2)}$$

Where 'S' is the solubility co-efficient of CO₂ in plasma:

0.03 mm/L/mmHg or 0.23 mm/L/kPa. PCO₂ can therefore be measured in mmHg or kPa; HCO₃⁻ is measured in mmol/L or mEq/L.

Disorders in pH are classified as either primarily metabolic or respiratory in origin. If, for example, the primary disorder is respiratory in origin, then an expected metabolic compensation in an attempt to normalise blood pH should occur, and vice versa where respiratory compensation should occur if an acid-base disorder is of metabolic origin (Hopper 2014a; Irizarry & Reiss 2009; Monnig 2013). A mixed disorder may be present when the expected compensation is not present. Mixed disorders, however, can be additive (where the respiratory and non-respiratory/metabolic disturbances both alter the pH in the same direction) or off-setting (where the respiratory and non-respiratory/metabolic disturbances alter the pH in opposing directions) (Bateman 2008; Hopper 2014b; Irizarry & Reiss 2009; Monnig 2013).

The traditional approach can be further expanded by analysing the BE and AG (Hopper et al. 2014a). The BE (also known as the Actual Base Excess, ABE, or BE_b, where b denotes blood), is the amount of strong acid or base that must be added to titrate 1 L of blood to a pH of 7.4 at 37 °C while the PCO₂ is held constant at 40 mmHg (Bateman 2008). The BE, derived by the van Slyke equation, is used to determine the contribution of non-respiratory factors to acid-base disturbance (Hopper et al. 2014a; Monnig 2013; Siggaard-Andersen 1977). The BE defined by the van Slyke equation is accurate in vitro calculations but inaccurate when applied in vivo due to the variations in HCO₃⁻ with changes in PCO₂. The standard base excess (SBE, or BE_{ecf}, where ecf denotes extracellular fluid) was therefore developed, which incorporates the effects of haemoglobin as a buffer as well as the equilibration of HCO₃⁻ across the extracellular space (Hopper & Haskins 2008; Kellum 2005). The AG is based upon the preservation of plasma electroneutrality in order to quantify unmeasured ions which can further assist in determining the cause of a non-respiratory acid-base disturbance (Hopper et al. 2014b; Monnig 2013).

The physicochemical approach described by Stewart, aims to identify complex disturbances by analysing three independent variables: the PCO₂, the strong ion difference (SID) and the total quantity of non-volatile weak acids (A_{TOT}) (Hopper et al. 2014a). Strong ions are completely dissociated at physiological pH and the SID is the arithmetic difference between the plasma

concentrations of strong cations and anions. The A_{TOT} considers the two main plasma weak acids: albumin and phosphate (Hopper et al. 2014a). The SID can be calculated in two ways: the apparent SID (SIDa) which considers measured cations and chloride concentrations; or the effective SID (SIDe) which considers HCO₃⁻ and A_{TOT}. In the healthy patient, the arithmetic difference between SIDa and SIDe (the strong ion gap, SIG) is close to zero. Increases in SIG are associated with unmeasured anions (Hopper et al. 2014b). Thus, using the Stewart approach, acid-base disturbances can be described in terms of changes of the PCO₂, SID and the A_{TOT}, with unmeasured anions quantified using the SIG (Torrente et al. 2014).

Lastly, the semiquantitative approach described by Fencel et al. (2000) combines the traditional and physicochemical approaches (Hopper et al. 2014a; Hopper et al. 2014b). Fencel et al. (2000) criticised the fact that the BE and AG methods do not consider the influence of non-bicarbonate buffers, namely albumin and phosphate on metabolic acid-base abnormalities. The semiquantitative approach examines the individual effects of free water, chloride, albumin, phosphorus and lactate on the BE. Using this method, unmeasured anions (XA) are quantified by the difference between the BE and sum total of the individual effects (Hopper et al. 2014a).

The major advantage of the traditional approach to blood pH analysis is its wide recognition as well as its relative simplicity of application (Monnig 2013). However, critics of the traditional approach claim that its main disadvantage is that it is descriptive, rather than offering a mechanistic explanation for changes in blood pH (Monnig 2013). Furthermore, it has been found to be too simplistic to identify complex mixed disturbances commonly found in critical patients (Hopper et al. 2014a). Moreover, Hopper et al. (2014a) criticised the traditional approach in its failure to provide a clear managemental path for critical patients.

The physicochemical and semiquantitative approaches have been found to be more effective at identifying acid-base abnormalities and quantifying unmeasured anions in dogs and cats presenting for emergency care, when compared to the traditional approach (Hopper et al. 2014a; Hopper et al. 2014b). Similarly, in horses, the physicochemical approach has been shown to be superior at detecting acid-base abnormalities resulting from electrolyte imbalances after cross-country events and gastrointestinal disorders (Gomez et al. 2013; Kirsch & Sandersen 2020; Navarro et al. 2005). The physicochemical approach has also been shown to be better at detecting acid-base disorders in cattle suffering various gastrointestinal and respiratory disorders (Garzon-Audor et al. 2020). However, these approaches require extensive post-analytical calculations and are thus prone to amplification of errors accumulated

during the pre-analytical and analytical phases. Furthermore, these approaches are cumbersome and difficult to apply at the patient-side, without using computer spreadsheets (Hopper et al. 2014a; Monnig 2013). Hopper et al. (2014a) has suggested that these approaches may not improve the management of all patients and thus should only be applied to critically ill patients (Hopper et al. 2014b).

The differences in PCO_2 , electrolyte and albumin reference ranges for domestic species must be borne in mind, as these will influence reference ranges for variables relating to all three approaches to acid-base analysis (PCO_2 , AG, A_{TOT} , SID, sum total of effects) (Dugdale 2010; Hopper et al. 2014a; Hopper et al. 2014b). The chemical characteristics of non-volatile weak acids in plasma pose an additional layer of complexity. The A_{TOT} value for a species is directly derived from the effective dissociation constant of non-volatile weak acids in plasma (McCullough & Constable 2003). McCullough and Constable (2003) demonstrated that the effective dissociation constant of these weak acids is similar between humans, cattle and cats but significantly different in horses. Therefore, particular attention must be paid to the species at hand when acid-base analysis is carried out.

It is crucial to ensure that the results obtained are those of the patient being assessed. Some sources recommend attaching at least two patient identifiers to the blood gas syringe before submission for analysis (Dukić et al. 2016). This strategy should reduce the likelihood of confusing patient samples during

analysis, especially during busy periods in the laboratory. It is also prudent to record the patient's physical condition and clinical parameters at the time of sample collection (Baird 2013) so that the results can be interpreted in context of the patient's physiology. Furthermore, understanding the patient's physiological state will assist in deciding whether to collect an arterial or venous sample. Additionally, laboratory results should be reported in a timeous manner in order to optimise patient outcomes, especially unstable patients whose condition may quickly deteriorate without rapid interventions which are based on laboratory results.

In 2016, Roels et al. compared the results of four different analysers in healthy dogs and dogs with cardiopulmonary disease. Significant differences in the pH, PO_2 and PCO_2 values between analysers were noted. Therefore, in institutions where analysers from more than one manufacturer are present, it is recommended that serial blood gas analyses for a patient be performed on the same analyser (Roels et al. 2016).

Conclusion

The sources of error during blood gas analysis, as summarised in Table III, are numerous. However, the majority of these errors occur during the pre-analytical phase as a direct result of human error. It is imperative that the veterinarian performing or requesting the test understands the indications for blood gas analysis, anaerobically aspirates the sample into a syringe containing an

Table III: A summary of the sources of error and their consequences during blood gas analysis

Error	Consequences
Pre-analytical phase	
Test over-utilisation	Iatrogenic anaemia
Entrainment of air bubbles	PO_2/PCO_2 equilibration with environment False hypocalcaemia
Storage on ice	PO_2 equilibration with environment
Excessive time to analysis	Significant deviations in PO_2/PCO_2
Excessive/incorrect heparin	Analyte dilution Deviations in cation measurements
Aspiration of sample from IV fluid containing catheter	Dilution of analytes Falsely elevated or reduced lactate level depending on fluid type
Haemolysis	False hyperkalaemia, hyponatraemia, hypocalcaemia
Transport via pneumatic tube system	PO_2/PCO_2 equilibration with air bubbles Haemolysis
Analytical phase	
	Amplification of errors from previous phase
Improper QC/maintenance	Unreliable results
Electrolytes with ionisation similar to standard electrolytes	Falsely elevated electrolyte with similar ionisation
Antifreeze intoxication	False hyperlactataemia
Aspirin intoxication	False hyperchloraemia
Post analytical phase	
	Amplification of errors from previous phases
Use of different analysers for serial analysis	Improper trends in pH, PO_2 , PCO_2
Application of traditional approach (Henderson-Hasselbalch)	Amplification of errors from previous phases Too simple to identify complex acid-base disturbances
Application of physicochemical and semiquantitative approaches (Stewart and Fencil)	Amplification of errors from previous phases Cumbersome May only be applicable to critical patients
Use of incorrect reference range for species	Incorrect interpretation of results

PCO_2 – carbon dioxide tension, PO_2 – oxygen tension, IV – intravenous, QC – quality control

appropriate quantity of the correct anticoagulant and correctly identifies the sample. A thorough history and clinical exam as well as appropriate QC and analyser maintenance should mitigate most of the potential analytical errors. Understanding how each blood gas test variable was obtained should provide insight of the potential drawbacks of each approach to blood gas analysis.

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