Electrolyte analysis in dogs with hypoadrenocorticism: A comparison of two in-house analysers with a reference laboratory

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Abstract

Dog's treated for hypoadrenocorticism are routinely monitored through analysis of their blood electrolyte concentrations. This is often performed with point-of-care analysers to facilitate faster medication dose adjustments based on the results.

The objective of this study was to investigate the performance of two point-of-care analysers (IDEXX Catalyst™ Dx and IDEXX VetStat®) against a reference laboratory indirect ion-selective-electrode method (Olympus AU640 or Siemens Dimenson Xpand Plus) for the measurement of blood sodium, potassium and chloride concentrations, as well as the sodium: potassium ratio, in dogs diagnosed with and treated for hypoadrenocorticism.

Forty-eight dogs were enrolled into a prospective cross-sectional study. Paired blood samples were taken and tested on the two point-of-care analysers and at the reference laboratory. Statistical analysis was then performed with Spearman's correlations, Bland-Altman analysis, Passing-Bablok regression and Cohen's Kappa analysis. The clinical effects of inaccurate electrolyte analysis were also considered.

In total, 329 samples were tested on the IDEXX Catalyst™ Dx analyser, whilst another 72 samples were tested on the IDEXX VetStat®. Spearman's correlations were acceptable for all electrolytes, but regression analysis identified both proportional and constant bias for some analytes. There was poor agreement between sodium and chloride concentrations on both analysers and they tended to give higher results than the reference ISE method for all analytes, except for potassium when measured on the IDEXX VetStat®.

In conclusion, there are inherent differences between the electrolyte concentrations measured by these two point-of-care analysers and reference laboratory methods in dogs with hypoadrenocorticism. Clinicians should be aware of this and consistent in their method of measurement of electrolytes when monitoring these dogs

Key words: *canine, dogs, hypoadrenocorticism, Addison's disease, fludrocortisone, desoxycortone pivalate, DOCP, electrolytes, sodium, potassium, chloride, Olympus AU640, Siemens Dimension Xpand Plus, Idexx Catalyst™ Dx, Idexx VetStat®*

Abbreviations:

Acute Kidney Injury (AKI)
Adrenocorticotropic hormone (ACTH)
Angiotensin Converting Enzyme (ACE)
Allowable Total Error (TE_A)
American Society of Veterinary Clinical Pathology (ASVCP)
Antidiuretic Hormone (ADH)
Area Under Curve (AUC)
Arginine Vasopressin (AGP)
Atrial Natriuretic Peptide (ANP)
Bicarbonate (HCO_3^-)
Capillary Refill Time (CRT)
Chloride (Cl^-)
Cholecystokinin (CCK)
Chronic Kidney Disease (CKD)
Clinical Laboratory Improvement Amendment (CLIA)
Coefficient of Variation (CV)
Control Product (CP)
Corticotrophin releasing hormone (CRH)
Desoxycortone pivalate (DOCP)
Electrocardiography (ECG)
Electrogenic Sodium Channels (ENaC)
Enzymatic spectrophotometry (ES)
European Veterinary Endocrine Quality Assurance Scheme (EVE-QAS)
Ethylenediaminetetraacetic acid (EDTA)
Extracellular Fluid (ECF)
Haematocrit (HCT)
Hyperadrenocorticism (HAC)
Hypoadrenocorticism (HA)
Hypothalamic Pituitary Adrenal (HPA)
Inflammatory Bowel Disease (IBD)
Investigational product (IVP)
Intracellular Fluid (ICF)
Ion-selective electrode (ISE)
Limits of Agreement (LOA)
Observed Total Error ($TE_{(Obs)}$)
Packed Cell Volume (PCV)
Point-of-care (POC)
Potassium (K^+)
Polyuria (PU)
Polydipsia (PD)
Protein Losing Enteropathy (PLE)
Reference Intervals (RIs)
Renin-Angiotensin System (RAS)
Revolutions per minute (RPM)
Sodium (Na^+)
Sodium-Potassium Adenosine Triphosphatase (Na^+, K^+-ATPase)
Urine Specific Gravity (USG)
Zona Fasciculata (ZF)
Zona Glomerulosa (ZG)
Zona Reticularis (ZR)

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Dedication

For Boris, Cameo, Chintz, Daisy, Domino, Kedi, Lily, Maisie, Oscar, Pusscat, Rainbow, Ruby, Sinbad, Tory, Vanilla and YoYo; who together taught me about love, friendship, loyalty and unfortunately loss. I am a better vet and human being because of you all.

Declaration

I, Samuel Jack Fowlie, declare that the work in this thesis is original, was carried out solely by myself or with due acknowledgements. It has not been submitted in any form for another degree or professional qualification.

Printed Name: Mr Samuel Fowlie

Signature: …….

List of Publications

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1 Introduction

1.1 Hypoadrenocorticism

Hypoadrenocorticism (HA), also known as Addison's disease, is a well-described endocrinopathy in dogs characterised by a lack of adrenocortical function resulting in inadequate secretion of both mineralocorticoids (aldosterone) and glucocorticoids (cortisol). The anatomy and physiology of the adrenal glands is described in detail elsewhere (Dyce et al., 2017). The development of clinical signs is believed to require destruction of at least 90% of the adrenal cortex (Scott-Moncrieff, 2015).

1.1.1 Glucocorticoids

The major glucocorticoid is cortisol which is synthesised from cholesterol and secreted via a complex process involving multiple enzymes. Cortisol levels are regulated by the hypothalamic-pituitary-adrenal (HPA) axis via corticotrophin-releasing hormone (CRH) which is released from the hypothalamus and stimulates secretion of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. ACTH is released into the bloodstream and stimulates the adrenal gland cortex (zona fasciculata (ZF) and zona reticularis (ZR)) to produce cortisol. CRH release is triggered by pain, stress hypoglycaemia and physical exercise. As cortisol levels rise, CRH and ACTH release is inhibited via negative feedback and increased levels of ACTH also inhibit CRH release itself (Klein, 2013, Scott-Moncrieff, 2015). ACTH levels in humans fluctuate with circadian rhythms however this is not the case in the dog. In addition to CRH, ACTH release can also be stimulated by arginine vasopressin (AGP), angiotensin II, cholecystokinin (CCK), atrial natriuretic factor (ANP) and vasoactive peptides (Stewart and Krone, 2011).

Cortisol affects almost every tissue and is crucial to maintaining homeostasis. It is involved in the metabolism of sugars, fats and protein, improves the body's defence against long term stress by suppressing the immune system, reduces the release of inflammatory mediators and maintains the gastrointestinal mucosa (Peterson et al., 1996, Langlais-Burgess et al., 1995). Cortisol also helps to regulate blood pressure, water balance and vascular volume by increasing water diuresis, glomerular filtration rate and renal plasma flow from the kidneys. Cortisol binds to mineralocorticoid receptors but has only weak mineralocorticoid activity (Rose and Post, 2017).

1.1.2 Mineralocorticoids

The major mineralocorticoid is aldosterone. The zona glomerulosa (ZG) is the only layer of the adrenal cortex capable of secreting aldosterone as it contains the enzyme aldosterone synthase (P450 c11AS). Additionally it does not contain 17-alpha-hydroxylase (P450 c17) and as such, cannot synthesise cortisol (Scott-Moncrieff, 2015). Unlike the glucocorticoids, tropic hormones from the pituitary gland do not control mineralocorticoid release, instead aldosterone synthesis and secretion is controlled primarily by the renin-angiotensin system (RAS) and plasma potassium concentration and to a lesser extent by plasma sodium and ACTH concentrations (Klein, 2013). Angiotensin synthesis is stimulated by decreased extracellular fluid (ECF) volume and causes renin to be released from the juxtaglomerular cells in the kidney. Renin acts to convert angiotensinogen to angiotensin I which is in turn converted to angiotensin II primarily in the lungs by angiotensin converting enzyme (ACE). Angiotensin II stimulates increased aldosterone synthesis. Increased plasma potassium acts directly on the adrenal gland to promote aldosterone release (Scott-Moncrieff, 2015). An absence of ACTH decreases aldosterone secretion but does not prevent aldosterone secretion altogether as evidenced by normal mineralocorticoid levels in dogs post hypophysectomy (Meij et al., 1997).

Aldosterone promotes distal renal tubular reabsorption of sodium and chloride and facilitates the secretion of potassium and hydrogen ions via the kidney, sweat and salivary glands and the intestinal tract. As such a lack of aldosterone leads to hyponatraemia and hyperkalaemia, is critical in conserving body salts and can cause a mild metabolic acidosis (Scott-Moncrieff, 2015). Aldosterone release is inhibited by dopamine and ANP, which are released in response to volume expansion (DiBartola, 2012).

1.2 Aetiology of Hypoadrenocorticism

1.2.1.1 Primary Hypoadrenocorticism

1.2.1.1.1 Naturally Occurring Primary Hypoadrenocorticism

Naturally occurring primary hypoadrenocorticism results from atrophy or destruction of all three adrenal cortical layers. Primary immune-mediated destruction is thought to be the main cause and anti-adrenal antibodies have been detected in dogs (Schaer et al., 1986). Histological evaluation of the adrenal glands of dogs with primary hypoadrenocorticism typically shows lymphoplasmacytic inflammation (Frank et al., 2013). However, in most dogs a definitive cause is never proven, and their disease is considered idiopathic. Rare cases of adrenal cortex destruction with different aetiologies have also been reported such as infiltrative fungal or granulomatous disease, neoplasia, amyloidosis, infarction, haemorrhage due to trauma or coagulopathy, or iatrogenic causes (Labelle and De Cock, 2005, Kook et al., 2010, Korth et al., 2008, Rockwell et al., 2005, Reusch et al., 2007, Frank et al., 2013, Scott-Moncrieff, 2015).

1.2.1.1.2 Iatrogenic Primary Hypoadrenocorticism

Iatrogenic primary hypoadrenocorticism can result from drugs that cause destruction of the adrenal cortices. Mitotane (Lysodren) is directly cytotoxic to the adrenal gland and dogs may develop adrenocortical insufficiency during treatment for hyperadrenocorticism (HAC). This is usually transient however up to 5% of dogs may develop permanent hypoadrenocorticism (Kintzer and Peterson, 1991, den Hertog et al., 1999). Trilostane (Vetoryl) may cause adrenal suppression which is usually reversible but permanent hypoadrenocorticism has been reported associated with adrenal necrosis and haemorrhage (Reusch et al., 2007, Chapman et al., 2004, Reine, 2007, Braddock et al., 2003, Alenza et al., 2006, Neiger et al., 2002).

1.2.1.1.3 Atypical Primary Hypoadrenocorticism

Atypical hypoadrenocorticism represents <10% of cases. Affected dogs have normal electrolytes at diagnosis and are thought to have ZG sparing and therefore maintenance of normal aldosterone levels (Kintzer and Peterson, 1997a, Klein and Peterson, 2010a, Frank et al., 2013). However, some dogs with low aldosterone concentrations have also been found to have normal electrolyte concentrations implying that the maintenance of normal levels is not solely aldosterone dependent and there are other mechanisms by which dogs maintain normal electrolyte status (Baumstark et al., 2014b). It may be that the loss of adrenocortical tissue affecting the ZF and ZR occurs prior to or at a faster rate than the loss of the ZG and these patients may develop electrolyte abnormalities in time as their disease progresses (Klein and Peterson, 2010a, Scott-Moncrieff, 2015).

1.2.1.2 Secondary Hypoadrenocorticism

Iatrogenic secondary hypoadrenocorticism is much more common than naturally occurring disease. Typically, it is due to chronic administration and sudden withdrawal of exogenous glucocorticoids which suppress ACTH release from the anterior pituitary causing atrophy of the ZF and ZR. Alternatively iatrogenic disease may occur after hypophysectomy (Scott-Moncrieff, 2015).

Naturally occurring secondary hypoadrenocorticism is rarely reported and results from a failure of the pituitary gland to secrete ACTH or decreased CRH secretion by the hypothalamus (Kintzer and Peterson, 1997a). This may be due to destruction of the pituitary gland by neoplasia, inflammation, infection, infarction or head trauma and can cause acute neurological dysfunction and death (Platt et al., 1999, Foley et al., 2009, Eckersley et al., 1989, Scott-Moncrieff, 2015, Bertolini et al., 2007). Lack of ACTH causes severe atrophy of the ZF and ZR but leaves the ZG intact. As such serum electrolytes remain normal as aldosterone secretion is not affected resulting in a pure glucocorticoid deficiency. Unlike atypical primary hypoadrenocorticism, ACTH concentrations are low (Hess, 2017). Among all dogs with hypoadrenocorticism, the incidence of secondary hypoadrenocorticism is estimated between 4% and 24% (Klein and Peterson, 2010a).

1.2.1.3 Tertiary Hypoadrenocorticism

Tertiary hypoadrenocorticism is caused by hypothalamic disorders disrupting CRH secretion resulting in decreased ACTH production and is very rarely reported in humans and has never been reported in dogs (Klein and Peterson, 2010a).

1.3 Investigation of Hypoadrenocorticism

1.3.1 Historical and Clinical Signs of Hypoadrenocorticism

1.3.1.1 Signalment

Hypoadrenocorticism can affect dogs of any age, breed or sex however there are strong breed predispositions. Affected animals tend to be young to middle-aged with a median age at diagnosis of 4 years and a reported range of 2 months to 16 years (Peterson et al., 1996, Seth et al., 2011, Adler et al., 2007, Klein and Peterson, 2010a). Hypoadrenocorticism has been estimated to have a prevalence of up to 0.28% (Kelch et al., 1998) in the overall canine population. Any breed may be affected but certain breeds are at an increased risk for developing the disease. It is an autosomal recessive trait in the Standard Poodle (Famula et al., 2003), Portuguese Water Dog (Oberbauer et al., 2006) and Nova Scotia Duck Tolling Retriever (Hughes et al., 2007). In Bearded Collies, hypoadrenocorticism is a highly heritable trait but the exact mechanism of inheritance is unclear (Oberbauer et al., 2002). Females tend to be overrepresented, accounting for around 70% of cases however in these four breeds the female predisposition is not seen (Scott-Moncrieff, 2015). Nova Scotia Duck Tolling Retrievers also seem to have an earlier onset of disease compared to other breeds with an average age of 2.6 years (Hughes et al., 2007). Other breeds which are overrepresented include Great Danes, West Highland White Terriers, Springer Spaniels, Soft-Coated Wheaten Terriers and Basset Hounds (Klein and Peterson, 2010a, Thompson et al., 2007). Golden Retrievers, Yorkshire Terriers, Pit Bulls, Chihuahuas and Lhasa Apso’s have decreased risk (Peterson et al., 1996).

1.3.1.2 History and Clinical Signs

The onset of clinical signs can be gradual and may initially be missed by owners although in other cases a stressful event can precipitate illness. A history of episodic disease which responds to supportive care is suspicious for hypoadrenocorticism. Dogs with hypoadrenocorticism may exhibit a range of clinical signs, many of which are vague and non-specific and can be attributed to multiple body systems and differential diagnoses. Common clinical signs include anorexia, weight loss, vomiting, diarrhoea, lethargy, weakness and shaking/shivering. Polyuria (PU), polydipsia (PD) and abdominal pain may be observed and in more extreme cases affected dogs may have gastrointestinal haemorrhage, collapse or they may seizure due to hypoglycaemia. Glucocorticoid deficiencies may account for most of these signs due to their role in maintaining the gastrointestinal mucosa, blood pressure and fluid volume, body temperature and glucose levels. However when mineralocorticoid deficiencies are also present clinical signs such as PU/PD, hypovolemic shock, collapse and dehydration tend to be more severe (Greco, 2007, Peterson et al., 1996, Klein and Peterson, 2010a, Feldman et al., 2015)

1.3.1.3 Clinical examination

Physical examination is often normal especially in chronic cases and any abnormalities maybe vague and nonspecific. When abnormalities are present, they can range from mild dehydration to hypovolaemic shock and collapse with prolonged capillary refill time (CRT) and weak pulses. Most commonly dogs present with weakness, lethargy, hypothermia and dehydration. Dogs with hypoadrenocorticism are often in thin body condition and occasionally have abdominal pain. Bradycardia in a collapsed or unwell dog is suspicious for hyperkalaemia of which hypoadrenocorticism is a differential diagnosis (Klein and Peterson, 2010a, Greco, 2007).

1.3.1.4 Differential diagnosis

Given the vague and sometime episodic clinical signs which respond quickly to symptomatic treatment, HA can be confused for many other disease processes. These include intestinal disease (e.g. inflammatory bowel disease (IBD), protein losing enteropathy (PLE), parvovirus), kidney disease (e.g. acute kidney injury (AKI), chronic kidney disease (CKD)), endocrine disease (e.g. insulinoma, pheochromocytoma) and hepatic disease (e.g. chronic hepatopathy).

1.3.2 Clinical Pathological Changes Associated with Hypoadrenocorticism

1.3.2.1 Haematology

There are several non-specific haematological changes found in dogs with hypoadrenocorticism. The "classic" change is a reverse stress leucogram (i.e. low/normal neutrophil numbers with increased lymphocytes and eosinophils) but this is not present in most cases (Scott-Moncrieff, 2015). More commonly dogs with hypoadrenocorticism there is an absence of a stress leucogram in a systemically unwell dog but this is not specific enough to confirm a diagnosis (Seth et al., 2011, Zeugswetter and Schwendenwein, 2014). This change is due to a lack of cortisol which prevents sequestration of lymphocytes in the lymph nodes and bone marrow resulting in normal to elevated lymphocyte numbers in the circulation. One study found a lymphocyte count $>0.75x10^3/\mu L$ had a sensitivity of 100% in dogs with hypoadrenocorticism and a specificity of 35% (Seth et al., 2011). An eosinophilia is also not uncommonly found, occurring in around 10 to 20% of cases (Greco, 2007, Kintzer and Peterson, 1997a, Klein and Peterson, 2010a). Another common finding (21% - 25% of cases) is a normocytic normochromic non-regenerative anaemia which is typically mild to moderate (with a haematocrit (HCT) of 20%-35%) although this can be more severe. This anaemia is attributed to a lack of red cell production due to cortisol deficiency combined with gastrointestinal blood loss. Melena or haematochezia is present in up to 15% of dogs (Medinger et al., 1993, Peterson et al., 1996). This anaemia may be masked by dehydration and some dogs may even appear to have increased an increased packed cell volume (PCV) if the dehydration is severe. As with clinical signs, haematological findings may be normal. Dogs with glucocorticoid deficient hypoadrenocorticism (i.e. with normal electrolytes) are more likely to be anaemic than those with mineralocorticoid deficiency alone (Thompson et al., 2007).

1.3.2.2 Electrolytes and the Sodium: Potassium Ratio

Electrolyte abnormalities are the most common biochemical finding in dogs with hypoadrenocorticism and their measurement is the focus of this MVM thesis. They are discussed in greater detail in chapter 1.4.

1.3.2.3 Biochemistry

Most canine patients with hypoadrenocorticism are azotaemic at presentation due to hypotension, reduced renal perfusion and hypovolemia due to inadequate reabsorption of water from the kidneys. This may be marked and difficult to differentiate from AKI (Peterson et al., 1996). This azotaemia tends to resolve quickly with IV fluid therapy when treating HA in contrast to AKI. The urea maybe disproportionally more increased than creatinine in part due to gastrointestinal haemorrhage due to glucocorticoid deficiency and reduced gastrointestinal tract perfusion (Scott-Moncrieff, 2015).

Around a third of patients may be hypoglycaemic and this may be severe enough to cause seizures (Lifton et al., 1996). This is more common in dogs with atypical HA due to glucocorticoid deficiency, reduced gluconeogenesis and increased insulin sensitivity (Scott-Moncrieff, 2015).

Another third of patients have increases in their liver enzymes. This may be a combination of secondary reaction and hepatic dysfunction. Less commonly dogs may have hypoalbuminaemia (6 – 39%) and hypocholesterolaemia (7%). These changes are more frequently seen in atypical hypoadrenocorticism and are thought to be due to a combination of hepatic dysfunction (albumin and cholesterol), protein losing enteropathy (albumin) and altered fat metabolism (cholesterol) (Scott-Moncrieff, 2015).

Patients may have a mild metabolic acidosis due to reduced hydrogen ion excretion, hypovolaemia and hyperlactatemia as a result of poor tissue perfusion (Kintzer and Peterson, 1997b).

1.3.2.4 Urinalysis

Most dogs with hypoadrenocorticism are hypovolemic at diagnosis with a consequent pre-renal azotaemia. However, despite this, their urine specific gravity (USG) is often <1.030 as they are unable to conserve water.

1.3.2.5 Blood Pressure

Many dogs with hypoadrenocorticism are hypotensive at the time of initial examination with a median systolic blood pressure of 90 mmHg (range 40-150 mmHg) versus a median of 140 mmHg (range 50-210 mmHg) in dogs with other causes of illness (Seth et al., 2011).

1.3.2.6 Differential diagnosis

As with the clinical signs and physical examination findings, laboratory findings can be non-specific and confused with other disease processes. The classic electrolyte abnormalities of hyponatremia and hyperkalaemia and therefore low Na^+: K^+ ratios may be seen in dogs with gastrointestinal, renal or cardiac disease, urinary obstruction, body cavity effusions and diabetes mellitus. This can be compounded in patients with more than one concurrent illness. Additionally, gross lipemia and potassium EDTA contamination can cause artefactual changes in electrolyte concentrations.

1.3.3 Diagnostic Imaging

1.3.3.1 Radiography

Radiography is indicated to evaluate for other differential diagnoses such as obstructive gastrointestinal disease. Common thoracic and abdominal radiographic abnormalities in untreated dogs with hypoadrenocorticism are non-specific and secondary to hypovolaemia. They include microcardia, microhepatica, narrow caudal vena cava and small cranial lobar pulmonary artery (Melian et al., 1999, Peterson et al., 1996). Less commonly there may be evidence of megaoesophagus or oesophageal dilation which is reversible with treatment (Bartges and Nielson, 1992, Whitley, 1995, Lifton et al., 1996, Peterson et al., 1996, Kintzer and Peterson, 1997a). The cause of this is not clear but may be due to cortisol deficiency and associated muscle weakness or due to abnormal sodium and potassium concentrations affecting membrane potentials and neuromuscular function. However, abnormal serum electrolyte concentrations are not always documented (Scott-Moncrieff, 2015).

1.3.3.2 Echocardiography

Dogs with hypoadrenocorticism are sometimes assessed with echocardiography due to bradycardia and concerns about heart function such as hypotension. They may have poor systolic function and appear volume underloaded which can be confused for heart disease e.g. dilated cardiomyopathy. These changes improve with treatment of the underlying hypoadrenocorticism (Spence et al., 2018).

1.3.3.3 Abdominal ultrasound

Most dogs with hypoadrenocorticism have measurably decreased adrenal gland size and occasionally they cannot be visualised by ultrasonography. A left adrenal gland thickness <3.22 mm is strongly suggestive of HA however the adrenal glands may be normal in size (Hoerauf and Reusch, 1999, Wenger et al., 2010). Ultrasonographic examination also allows investigation of other differential diagnoses which can all present with similar clinical signs.

1.3.4 Electrocardiography

Many dogs with hypoadrenocorticism are bradycardic on presentation and therefore may be assessed via electrocardiography (ECG). This bradycardia is due to disturbed cardiac function as a result of hyperkalaemia which if left untreated can result in ventricular fibrillation, standstill and cardiac arrest (Melian and Peterson, 1996). This occurs as a result of a decreased ratio between the potassium concentration in the intracellular fluid (ICF) and ECF resulting in a decrease in the resting membrane potential. Persistent depolarisation inactivates cell surface Na^+ channels reducing excitability and thus muscle weakness and abnormal cardiac conduction occur (Scott-Moncrieff, 2015). The severity of bradycardia is not precisely correlated with serum potassium levels but ECG changes generally worsen as the concentration rises (Côté and Ettinger, 2017). Characteristic changes consistent with hyperkalaemia are seen on ECG, namely low amplitude or absent P waves, spiked T waves, widened QRS complexes and ectopic ventricular beats. ECG changes however are not always present or consistent with other factors affecting ECF K^+ concentrations such as pH, hyponatraemia and the presence of hypercalcaemia which may be cardioprotective. Occasionally other less common arrhythmias such as atrial fibrillation are seen due to other concurrent electrolyte abnormalities, acidosis and myocardial reperfusion injury (Riesen and Lombard, 2006).

1.3.5 Confirming the Diagnosis of Hypoadrenocorticism

1.3.5.1 Basal Cortisol

Basal (or "resting") cortisol levels are an excellent screening test to exclude hypoadrenocorticism and various cut-off levels have been investigated in the literature. Most clinicians currently use a cortisol level >55nmol/L to exclude hypoadrenocorticism due to its sensitivity of 100%, however it is only 63 – 78% specific. A lower cut-off of 28 nmol/L was previously used due to its higher specificity of 92 – 98% but its sensitivity may be as low as 86% (Bovens et al., 2014, Lennon et al., 2007). More recently a cut-off of 22 nmol/L has been suggested to maximise the sensitivity and specificity at 97 and 98% respectively (Gold et al., 2016). Cortisol remains stable in serum for up to 5 days (Behrend et al., 1998) and in dogs, its release is not affected by the time of day (Scott-Moncrieff, 2015). Cortisol assays are run on plasma by radioimmunoassay, chemiluminescent assay or enzyme-linked immunosorbent assay (Russell et al., 2007).

1.3.5.2 ACTH Stimulation Test

The ACTH stimulation test is the recognised standard for confirming a diagnosis of hypoadrenocorticism. It involves the measurement of a basal cortisol level, followed by an injection of synthetic ACTH, followed by a second cortisol measurement. Various different protocols have been examined in the literature comparing the route of administration of the synthetic ACTH (intravenous, intramuscular or perivascular) (Cohen and Feldman, 2012, Johnson et al., 2017), different doses of synthetic ACTH (0.5 µg/kg , 5 µg/kg, 250 µg/dog) (Martin et al., 2007, Lathan et al., 2008) and different timings for the second post-ACTH cortisol sample (30 to 90 minutes) (Frank et al., 2000).

These findings have recommended a standard dose of 5 µg/kg synthetic ACTH with a second post-ACTH sample taken 60 minutes later. A diagnosis of hypoadrenocorticism is made when both the pre and post ACTH cortisol levels are <55nmol/L, with a sensitivity and specificity of 100%. The use of the ACTH stimulation test and its confounding factors such as exogenous corticosteroid use, are reviewed in more detail elsewhere (Scott-Moncrieff, 2015, Klein and Peterson, 2010b, Spence et al., 2018).

1.3.5.3 Further Confirmatory Testing

Further information regarding the use of endogenous ACTH levels, aldosterone concentrations, plasma renin activity, aldosterone-to-renin ratio and the cortisol-to-creatinine ratio are reviewed elsewhere (Javadi et al., 2006, Scott-Moncrieff, 2015, Boretti et al., 2015, Lathan et al., 2014, Baumstark et al., 2014b, Thompson et al., 2007, Lifton et al., 1996, Baumstark et al., 2014a, Montori and Young, 2002, Boer et al., 1985).

1.3.6 Treatment of Hypoadrenocorticism

1.3.6.1 Acute Treatment of Hypoadrenocorticism

The acute management of hypoadrenocorticism is discussed in more detail elsewhere (Scott-Moncrieff, 2015, Spence et al., 2018). Most patients with hypoadrenocorticism present acutely due to dehydration, hypovolaemia and hypotension due to mineralocorticoid deficiency. Therefore, initial management is directed towards the administration of intravenous fluid therapy and management of electrolyte disturbances if present. This is one reason why accurate and reproducible results between electrolyte analysers is important. Care must be taken not to correct hyponatraemia too quickly due to the risk of causing delayed pontine osmotic demyelination and resultant neurological signs which can be fatal (O'Brien et al., 1994, Churcher et al., 1999). Hyperkalaemia will usually resolve with fluid therapy but often large volumes, so called “shock rates” of 40 to 80 ml/kg, may be needed. This is usually administered in smaller boluses of 5 to 10 ml/kg to effect with frequent reassessment of the dogs clinical status (Gunn et al., 2016). If hyperkalaemia does not resolve or if the patient is severely bradycardic with ECG changes, IV calcium gluconate can be given diluted and administered slowly intravenously. This reduces the excitability of the cardiac myocytes and is cardioprotective (DiBartola, 2001). The use of neutral insulin and dextrose to move potassium intracellularly, is often used to treat hyperkalaemia due to urinary obstruction and can be considered in hypoadrenocorticism cases. However, care must be taken not to worsen any existing hypoglycaemia (Scott-Moncrieff, 2015).

Glucocorticoid (plus or minus mineralocorticoid) supplementation is essential to treat an Addisonian crisis however samples for ACTH stimulation and other confirmatory testing should ideally be collected prior to administration of any exogenous steroids. This is because they can cause HPA axis suppression through negative feedback on the pituitary gland, suppressing cortisol release and may also cross react with commercial cortisol assays. Hydrocortisone sodium succinate has equal glucocorticoid and mineralocorticoid activity and has been shown to achieve faster normalisation of electrolytes and resolution of clinical signs other than steroids when administered as a constant rate infusion (0.5 – 0.625mg/kg/hour) (Gunn et al., 2016).

1.3.6.2 Chronic Treatment of Hypoadrenocorticism

All patients with hypoadrenocorticism require glucocorticoid supplementation regardless of if they have classical or atypical forms of the disease (Klein and Peterson, 2010b, Spence et al., 2018). Mineralocorticoid supplementation however is only required when there are electrolyte derangements or inappropriately low endogenous aldosterone levels (Baumstark et al., 2014b, Wakayama et al., 2017).

1.3.6.2.1 Glucocorticoids

Most patients receive glucocorticoid supplementation in the form of prednisolone given once a day. Prednisolone is a pure glucocorticoid so patients requiring mineralocorticoid support will require this separately as discussed below (Scott-Moncrieff, 2015). A wide range of doses are reported in the literature (<0.05 to 0.4 mg/kg/day) and the dose required seems to be vary between individuals (Kintzer and Peterson, 1997b). During physiological stress or illness, glucocorticoid requirements may increase by up to 5 fold (Klein and Peterson, 2010b). Prednisolone has the side effects of causing polyphagia, PU, PD, panting and poor hair growth (Plumb, 2018).

1.3.6.2.2 Mineralocorticoids

When necessary, there are two treatments commonly used for chronic aldosterone supplementation in dogs. Over and under dosing can cause hypertension and polydipsia, depression, lethargy and gastrointestinal signs (Plumb, 2018).

1.3.6.2.2.1 Fludrocortisone acetate

Fludrocortisone acetate is a steroid formulation with both glucocorticoid and mineralocorticoid activity and as such, adjunctive prednisolone is often not required however it does decrease the time for clinical stabilisation early in treatment (Roberts et al., 2016). The use of fludrocortisone is off license in dogs with a recommended starting dose of 0.02 mg/kg/day, given as one or two doses (Plumb, 2018), however a wide range of doses are reported (0.01 to 0.08 mg/kg/day) (Scott-Moncrieff, 2015, Kintzer and Peterson, 1997b, Roberts et al., 2016).

1.3.6.2.2.2 Desoxycortone pivalate

Desoxycortone pivalate (DOCP) is a pure mineralocorticoid (McCabe et al., 1995, Lynn and Feldman, 1991, Lynn et al., 1993, Van Zyl and Hyman, 1994). Although it may have a trivial effect on glucocorticoid receptors, additional glucocorticoid supplementation is required (Scott-Moncrieff, 2015). DOCP is the only licensed treatment of hypoadrenocorticism in dogs and two 25 mg/ml prolonged-release microcrystalline formulations are available; Percorten® (Novartis) in North America and Zycortal® (Dechra Ltd) in Europe. These have recently been shown to be non-inferior (Farr et al., 2020). The starting dose of Zycortal® is 2.2 mg/kg administered subcutaneously every 25 – 28 days (European Medicines Agency, 2019) although evidence from studies using Percorten® suggests that often much lower doses are required long term (Kintzer and Peterson, 1997b, Bates et al., 2013). Due to its rapid onset of action, only a brief transition period of around 48 hours is needed when changing from fludrocortisone to DOCP (Scott-Moncrieff, 2015, Ramsey et al., 2016) with peak activity around 10 days after injection (Jaffey et al., 2017). This is reflected in the electrolyte concentrations 10 – 14 days following injection and helps to inform about future dose adjustments (Mason et al., 2017). Electrolyte concentrations at 25 – 28 days give information on the duration of action (Lynn et al., 1993). Recent studies have investigated different dosing intervals to reduce the cost of treatment (Jaffey et al., 2017).

1.3.7 Monitoring of Hypoadrenocorticism

Basal cortisol and ACTH stimulation tests cannot be used to monitor dogs on treatment for hypoadrenocorticism as both prednisolone and fludrocortisone acetate cross-react with commercial cortisol assays (Krasowski et al., 2014). Dogs are primarily monitored by a combination of clinical signs (glucocorticoid) and electrolytes (mineralocorticoid).

Glucocorticoid dose decreases are warranted in patients with PU/PD, polyphagia, panting and a poor hair coat (i.e. clinical signs of glucocorticoid excess), whilst dose increases are necessary in patients that are lethargic, weak or having gastrointestinal signs (Scott-Moncrieff, 2015, Lathan and Thompson, 2018, Farr et al., 2020). Some overlap however exists with lethargy and weakness potentially indicating either glucocorticoid deficiency, mineralocorticoid deficiency or rarely, mineralocorticoid excess.

Mineralocorticoid supplementation (DOCP or fludrocortisone) acts to reduce potassium and increase sodium levels. Therefore, doses are reduced if hypernatremia or hypokalaemia occurs and increased with hyponatremia or hyperkalaemia. Therapy aims to maintain sodium and potassium levels within their normal limits (Lathan and Thompson, 2018, Adler et al., 2007). The sodium: potassium (Na^+: K^+) ratio has also been suggested as a clinical target for monitoring mineralocorticoid supplementation with a target of 27 – 32 given by the manufacturers of Zycortal® (European Medicines Agency, 2019). As discussed in chapter 1.4.4, whilst 27 has been shown to be a sensitive marker for mineralocorticoid deficiency and 40 is defined as the upper limit of "normal", 32 is a subjective choice with no evidence that it is any better than any other number in the normal range for discriminating well controlled from poorly controlled dogs (Adler et al., 2007, Scott-Moncrieff, 2015, Nielsen et al., 2008, Roth and Tyler, 1999).

Electrolytes are frequently measured "in-house" to allow for faster diagnoses and adjustments to treatment plans in a range of conditions not just hypoadrenocorticism. Different machines are frequently used between multiple branches of a practice (e.g. out of hours versus during the day) or even within a single building depending on what other tests required (e.g. blood gas analysis versus a full biochemistry with electrolytes).

More advanced monitoring through repeated measurement of endogenous ACTH concentrations, plasma renin activity and blood pressure are discussed further elsewhere (Spence, 2020, Kaplan and Peterson, 1995).

1.3.8 Prognosis

The long-term prognosis of primary HA in dogs is good provided a prompt diagnosis is made and the correct treatment is instituted. Most mortality occurs around the time of initial presentation in acute Addisonian crisis. The median time till stabilisation is 3 months however concurrent prednisolone administration results in faster stabilisation times (Roberts et al., 2016). Therefore, frequent electrolyte monitoring is essential, especially earlier in the course of disease. There is no link between sex, breed or age of diagnosis on the median survival time of 4.7 years (Kintzer and Peterson, 1997b).

1.4 Electrolytes

Electrolyte abnormalities, namely hyperkalaemia and hyponatraemia, are the classic clinicopathological abnormalities associated with primary hypoadrenocorticism (Peterson et al., 1996). They occur due to mineralocorticoid deficiency resulting in the kidneys being unable to conserve sodium (Na^+) or excrete potassium (K^+) (DiBartola and de Morais, 2012, DiBartola, 2012) . Hyperkalaemia occurs most commonly (74 – 96% of cases), followed by hyponatremia (56 – 100% of cases) and as such the Na^+: K^+ ratio is commonly evaluated and low (Lifton et al., 1996, Peterson et al., 1996, Sadek and Schaer, 1996, Thompson et al., 2007). On the other hand, some dogs with HA may have normal electrolyte concentrations including dogs with atypical HA, although not all dogs with normal electrolytes are atypical (Baumstark et al., 2014b). Isolated changes in sodium and potassium concentrations are more common in hypoadrenocorticism than changes in both electrolytes and up to a third of dogs may have electrolyte concentrations within the normal reference interval (Hughes et al., 2007).

Although hyponatremia and hyperkalaemia predominate, hyperphosphataemia (68%), hypochloraemia (42%) and hypercalcaemia (32%) may also occur (Peterson et al., 1996, Greco, 2007, Kintzer and Peterson, 1997a, Hess, 2017). These changes may occur together or independently and are likely due to altered electrolyte exchange within the kidneys as a result of both mineralocorticoid deficiency (chloride) and glucocorticoid deficiency (calcium/phosphate) (Adamantos and Boag, 2008, Gow et al., 2009).

1.4.1 Sodium

Sodium is the major extra extracellular cation and is a key determinant of plasma osmolality, tonicity and ECF volume. Changes in serum sodium often reflect changes in total body water stores rather than changes in total body sodium content themselves. ECF volume is maintained via sodium retention in response to antidiuretic hormone (ADH) and aldosterone acting on the kidney. The ECF sodium concentration is approximately 140 mEq/L and ICF is around 10 mEq/L (DiBartola, 2012).

1.4.1.1 Normal Sodium Homeostasis

Sodium is filtered by the glomeruli and reabsorbed by the renal tubules via active transport through sodium-potassium adenosine triphosphatase (Na^+, K^+-ATPase) pumps in the basolateral membranes of tubular cells. This translocates sodium from the tubular cell cytoplasm into the peritubular interstitium maintaining low ICF concentrations which in turn promotes sodium entry at the luminal surface. Around two thirds of the filtered sodium is reabsorbed isosmotically with water in the proximal tubules. In the early proximal tubules sodium is reabsorbed with co-transporters for glucose, amino acids and phosphate via luminal Na^+-H^+ antiporter. In the late proximal tubule, sodium is reabsorbed primarily with chloride due to luminal Na^+-H^+ and Cl^- - $anion^-$ antiporters working in parallel. Approximately a quarter of filtered sodium is reabsorbed in the loop of Henle, primarily in the thick ascending limb. This occurs via the Na^+-H^+ antiporter and Na^+-K^+-$2Cl^-$ cotransporters. Sodium and chloride are passively reabsorbed in the thin descending and ascending limbs. Another 5% of the filtered sodium is reabsorbed in the distal convoluted tubule and connecting segment. In the early distal tubule, sodium is reabsorbed via an Na^+-Cl^- cotransporters (the site of action of thiazide diuretics). The final ~3% of filtered sodium is reabsorbed in the collecting ducts via passive diffusion through Na^+ channels in the luminal membranes of principal cells. One of aldosterone's effects is to increase the number of these channels to increase sodium reabsorption (DiBartola, 2012).

1.4.1.2 Hyponatraemia in Hypoadrenocorticism

Aldosterone mediates changes in renal absorption of sodium in response to dietary intake by increasing the number and activity of open Na^+ channels in the principal cells of the collecting duct. Mineralocorticoid deficiency therefore results in urinary loss of NaCl and depletion of ECF volume. This volume depletion is a strong non-osmotic stimulus for vasopressin release and impairs water excretion with a subsequent increase in potassium levels (DiBartola, 2012).

1.4.2 Chloride

Chloride (Cl^-) is the major extracellular anion and plays a key role in determining osmolality, tonicity and ECF volume along with sodium. It accounts for approximately two thirds of the anions in plasma and is the major anion filtered and reabsorbed in the kidney. Its concentration often parallels that of sodium to achieve electroneutrality, but each is independently regulated. Additionally, Cl^- has a key role in renal acid base regulation along with bicarbonate (HCO_3^-). Normal plasma chloride concentrations are around 110 mEq/L in dogs and 120 mEq/L in cats (de Morais, 1992).

1.4.2.1 Normal Chloride Homeostasis

Chloride is gained and lost through the gastrointestinal and urinary systems. Even under normal conditions the chloride concentration of various gastrointestinal fluids is very variable. Chloride is secreted along with sodium into gastric secretions and is reabsorbed in the jejunum. The highest concentrations of chloride are found in the ileum which is relatively less permeable to ion uptake than the jejunum. Chloride may be reabsorbed here in exchange for HCO_3^-. The colon has the lowest chloride concertation as up to 90% of the sodium and chloride delivered to it, is reabsorbed. Chloride may also be secreted by the jejunum, ileum and colon (Dobbins, 1985). A loss or gain of free water or electrolyte rich fluids, will result in a proportional change the sodium and chloride concentrations. For example, severe secretory diarrhoea may result in an excessive loss of electrolytes via intestinal secretions combined with water retention due to the actions of ADH in response to hypovolaemia. This has the effect of "free water" gain.

The kidneys also play an important role regulating plasma chloride concentration with chloride the second most common ion in glomerular ultrafiltrate after sodium. Most of the filtered chloride is reabsorbed in the renal tubules and is actively and passively linked to sodium reabsorption. In the proximal tubule, 50-60% of the filtered chloride is reabsorbed by basolateral membrane K^+-Cl^- cotransporters. In the loop of Henle, reabsorption occurs transcellularly via Na^+-K^+-$2Cl^-$ transporters (the site of action of loop diuretics) with chloride being the rate limiting step. In the distal convoluted tubule Na^+-Cl^- cotransporters (thiazide sensitive) reabsorb chloride and in the collecting duct chloride absorption is linked to bicarbonate transport and paracellular pathways (de Morais and Biondo, 2012)

1.4.3 Potassium

Potassium is the major intracellular cation (along with Magnesium) and is essential for numerous physiological processes including enzymatic action, neuromuscular and cardiac conduction and routine cell function particularly in muscle and nerve cells. Most of the body's potassium (60-75%) is found within muscle cells and the remainder within the bones. Only around 5% of potassium is in the ECF and the concentration is tightly regulated as small changes can be life threatening and cause marked organ dysfunction. The inverse relationship is seen to sodium concentrations with ECF potassium concentrations of around 4 mEq/L and ICF concentrations of 140 mEq/L (DiBartola and de Morais, 2012).

1.4.3.1 Normal Potassium Homeostasis

Plasma potassium is tightly regulated as small changes can have marked effect on organ function and severe abnormalities are life threatening. Normal potassium levels are maintained through internal and external balance. External potassium balance is achieved by matching output via the kidneys with intake from the gastrointestinal tract and diet. Around 90% of potassium that is excreted is removed via the urine (kidneys) with the remainder via the gastrointestinal tract (colon) (Bourgoignie et al., 1985). Almost all ingested potassium is absorbed non-selectively in the stomach and small intestine. Uptake of potassium in the small intestine is passive whereas active transport in response to aldosterone occurs in the colon during potassium excretion. If dietary potassium is inadequate, renal extraction of potassium decreases and vice versa. Internal potassium balance is maintained by translocation of potassium between the ECF and ICF. Between 50% and 66% of ingested potassium is excreted via the kidneys within four to six hours and effective translocation is required to allow the kidneys time to excrete the remainder. This is largely dependent on endogenous insulin and catecholamines (primarily epinephrine) promoting potassium uptake by cells. Insulin binds to insulin substrate receptor -1 which causes uptake of potassium and glucose (via GLUT-4 receptors). Catecholamines promote the uptake of potassium through stimulation of Na^+, K^+-ATPase pumps primarily in the liver and skeletal muscle. Tissue necrosis can cause the release of intracellular potassium particularly when skeletal muscle is affected. Acid-base balance is also important in potassium distribution with shifts of potassium in and out of cells at different ECF pH levels. In general acidosis causes potassium ions to move from the ICF to ECF (i.e. hyperkalaemia) and alkalosis causes the reverse with potassium moving into cells (i.e. hypokalaemia). This is most pronounced with sudden shifts in plasma pH and mainly appears clinically relevant with metabolic acidosis (DiBartola and de Morais, 2012). Endotoxins can stimulate Na^+, K^+ ATPase pumps and cause insulin release both of which promote intracellular potassium shifts.

As discussed, the kidneys are the primary regulators of potassium balance. Potassium is filtered at the glomerulus and then most (~70%) is reabsorbed in the proximal tubule with water and sodium and by paracellular diffusion. This occurs via potassium channels in both the luminal and basolateral membranes and by basolateral K^+-Cl^- cotransporters. Most of the remaining potassium (~20%) is reabsorbed paracellularly in the ascending limb of the loop of Henle via K^+ channels in the luminal membrane. Some transcellular reabsorption of potassium occurs via luminal Na^+-K^+-$2Cl^-$ cotransporters and basolateral K^+ channels and K^+-Cl^- cotransporters (DiBartola and de Morais, 2012).

The remaining (~10%) potassium is delivered to the distal nephron where potassium excretion or absorption may occur depending on dietary potassium intake. In the distal convoluted tubule luminal surface there are Na^+- Cl^- (thiazide sensitive) and K^+-Cl^- cotransporters which result in the secretion of potassium and reabsorption of sodium. There are also basolateral Na^+, K^+-ATPase pumps which help to maintain low intracellular sodium concentration and high intracellular potassium concentrations, further facilitating potassium secretion and sodium reabsorption. In the connecting tubule and collecting duct, principal cells are rich in basolateral Na^+, K^+-ATPase which moves potassium from the blood into the renal tubular cells for excretion under the control of aldosterone. The luminal membranes contain electrogenic sodium channels (ENaC) which are also stimulated by aldosterone (and the site of action of spironolactone). Sodium movement through this channel makes the lumen negatively charged thus facilitating secretion of K^+ ions through luminal K^+ channels. Type A (α) intercalated cells are found in the connecting tubule, cortical collecting duct and outer medullary collecting duct. They contain a range of transporters to secrete H^+ ions and reabsorb K^+ and bicarbonate (HCO_3^-). Potassium is actively transported across the luminal membrane by H^+, K^+-ATPase and then diffuses down its concentration gradient through K^+ channels in the basolateral membrane. Type B (β) intercalated cells are found only in the cortical collecting duct and primarily secrete HCO_3^-. Potassium is able to be reabsorbed from the last portion of the outer and all of the inner medullary collecting duct via the paracellular route due to the concurrent absorption with water (DiBartola and de Morais, 2012).

1.4.3.2 Renal Excretion of Potassium

Excretion of potassium in the distal nephron is mainly controlled by sodium absorption in the distal tubules and aldosterone. Increased extracellular potassium concentrations further stimulate aldosterone release and potassium excretion without sodium absorption. Conversely hypokalaemia (e.g. with dietary deficiency) stimulates potassium resorption. Renal excretion of potassium increases with the distal tubule flow rate. Higher rates (e.g. with fluid therapy, diuretic therapy, osmotic diuresis), enhances potassium excretion via increased flushing out of potassium and increased absorption of sodium via ENaC. Decreased distal tubule flow rates (e.g. with hypovolaemia) lead to decreased sodium delivery to the distal nephron and reduced potassium excretion. Increased renal tubule electronegativity in the collecting tubules enhances potassium excretion. This may occur secondary to increased sodium levels or high concentrations of negatively charged molecules (e.g. ketones, penicillin antibiotics, HCO_3^-). Additionally, potassium excretion is decreased with acidemia and promoted during alkalemia. Acidosis reduces excretion and promotes resorption of potassium in the distal nephron due to decreased activity of the Na^+, K^+-ATPase pumps in principal cells and increased activity of the H^+/K^+ pump in the intercalated cells. Alkalaemia promotes potassium loss through intracellular shift in potassium in exchange for H^+ ions and by stimulating ENaC (DiBartola and de Morais, 2012).

1.4.3.3 Hyperkalaemia in Hypoadrenocorticism

Aldosterone release is stimulated via hyponatremia and hyperkalaemia and causes sodium absorption and potassium excretion from the kidney via increased activity of the basolateral Na^+/K^+ pump and ENaC, creating a negative luminal charge which promotes potassium excretion in exchange for sodium. Aldosterone also directly causes increased permeability of the luminal membrane to potassium, promoting excretion in the kidney and colon. Therefore, mineralocorticoid deficiency results in a gradually worsening hyperkalaemia due to decreased urinary excretion of potassium (DiBartola and de Morais, 2012).

1.4.4 Sodium: Potassium Ratio

The normal Na^+: K^+ ratio in dogs are said to lie between 27:1 and 40:1 (Scott-Moncrieff, 2015). As a result of hyponatraemia and hyperkalaemia the Na^+: K^+ ratio is often low in dogs with primary hypoadrenocorticism (Bartges and Nielson, 1992). A Na^+: K^+ ratio of 27:1 or less is found in 85-100% of cases and has been considered suggestive of hypoadrenocorticism whilst using 24:1 as a cut off improves detection rates with a specificity of up to 100% (Adler et al., 2007). However, many different conditions can cause electrolyte abnormalities and several studies have demonstrated the lack of specificity in the diagnosis of HA (Adler et al., 2007, Nielsen et al., 2008, Roth and Tyler, 1999, Scott-Moncrieff, 2015, Schaer et al., 2001, Seth et al., 2011). In dogs with low Na^+: K^+ ratios, HA was the cause in 13%-24% (Nielsen et al., 2008, Roth and Tyler, 1999) of cases depending on which cut off was used (24:1 vs 27:1). Many other diseases therefore can cause marked changes in the Na^+: K^+ ratio including urinary, gastrointestinal and cardiorespiratory disease as well as artefactual changes due to sample contamination with potassium ethylenediaminetetraacetic acid (EDTA). Additionally, it is worth noting that taking the upper and lower reference intervals from the reference laboratory used in this study, it is possible to generate a range of between 23.6 and 46.8 in dogs with "normal" sodium and potassium concentrations. It is therefore preferable to consider sodium and potassium concentrations independently in relation to their reference intervals (Nielsen et al., 2008).

1.5 Electrolyte Analysis

Serum, plasma and whole blood concentrations of these major electrolytes can be measured via several different methods (Schindler et al., 2018). Flame photometry (FP) is the recognised reference method although it is rarely used outside of a research setting due to inherent impracticalities in carrying out the test. Therefore, most commercial reference laboratories use indirect potentiometry by an ion-selective electrode (ISE) as this produces results comparable to FP and is much easier and cheaper to perform. Ideally all electrolyte measurements would be made using an indirect ISE method however this is not always realistic. So called "in-house" point-of-care (POC) analysers allow for faster electrolyte measurements and therefore faster diagnoses and adjustments to treatment plans. However, inaccurate POC electrolyte measurements may lead to delayed or inappropriate therapy which could be clinically detrimental to patients. Dogs receiving treatment for hypoadrenocorticism are typically monitored by analysis of their plasma, serum or whole blood concentrations of sodium, potassium and the Na^+: K^+ ratio in conjunction with their clinical signs. The chloride concentration [Cl^-] is usually assayed along with these other electrolytes on most POC analysers.

Many different POC electrolyte analysers are commercially available for veterinary use and most commonly employ either direct potentiometry by an ISE, enzymatic spectrophotometry (ES), optical fluorescence (OF) or a colorimetric assay. In this study, three different methods were used measured on four different machines. Two POC analysers, the IDEXX Catalyst™ Dx and IDEXX VetStat® Electrolyte and Blood Gas Analyser were compared to a reference laboratory indirect ISE method initially using an Olympus AU640 and latterly the Siemens Dimension Xpand® Plus.

1.5.1 Electrolyte Analysis Interference

Every electrolyte analysis method is vulnerable to interference from physiological abnormalities and contamination (e.g. potassium EDTA). High levels of lipid (hyperlipidaemia) or protein (hyperproteinaemia) may falsely decrease the electrolyte concentrations measured in samples. This may be important especially in dogs with other endocrinopathies such as hypothyroidism. Electrolytes in plasma are confined to the aqueous component which comprises around 93% of plasma volume and thus techniques such as FP and indirect ISE are affected by the excluded solid plasma components. This effect is usually small but is most apparent with sodium concentration as it is at a much higher concentration in the ECF than potassium. This can result in "pseudo-hyponatraemia", however it may occur with all electrolytes. Electrolyte analysis based on optical fluorescence or spectrophotometry as found on many POC analysers can be adversely affected by increased levels of bilirubin (hyperbilirubinaemia). Similarly, haemolysis and altered red cell stability (e.g. prolonged storage) may increase serum and plasma potassium concentrations through the release of intracellular potassium. Dogs with increased potassium on their red cells, such as Akitas, Shiba Inu, Shar-pei and some other Asian dog breeds and animals with marked reticulocytosis and thrombocytosis may cause "pseudo-hyperkalaemia" (Bernardini et al., 2009, Stockham and Scott, 2008, Scott et al., 2015, Schindler et al., 2018).

1.6 Electrolyte Analysers

1.6.1 Reference Laboratory Analysers

1.6.1.1 Olympus AU640

The Olympus AU640 (Beckman-Coulter Inc., Fullerton, CA, U.S.A) is a multi-parametric wet biochemistry analyser available for routine biochemical and electrolyte analysis. Electrolyte analysis is based on indirect potentiometry by an ion-selective electrode (ISE) method for sodium, potassium and chloride determination (Blanc et al., 2000). Indirect potentiometry involves sample dilution before analysis. This is important because endogenous interferents such as hyperlipidaemia or hyperproteinaemia may falsely decrease electrolyte concentrations as discussed above.

1.6.1.2 Siemens Dimension Xpand Plus

The reference laboratory used in this study switched their biochemical and electrolyte analysis to the Siemens Dimension Xpand Plus (Siemens Healthcare Ltd, Surrey, UK) during the study period following 4 weeks of quality control to ensure comparable results. During this time, samples were run on both the Olympus AU640 and the Siemens Dimension Xpand Plus. Biochemical analysis used the same reagents and electrolyte analysis employed the same indirect ISE methodology on both machines. Due to the laboratory being unable to give an exact date for the switch between machines, these were treated as one indirect ISE method for the whole trial.

1.6.2 Point-of-care Analysers

1.6.2.1 IDEXX Catalyst™ Dx

The IDEXX Catalyst™ Dx analyser (IDEXX Laboratories, Inc., Westbrook, Maine, U.S.A) is one of the most common POC analysers used in veterinary clinics. It uses "dry-slide technology" for biochemical and electrolyte analysis. These slides are made up of several layers, which spreads the sample evenly, removes impurities and then reacts with the sample with a reagent before it is read by spectral analysis. This process involves an ionophore-bound fluorescent dye which reacts with the electrolytes. This produces fluorescence that is measured by ion-specific sensors which is converted to a numerical result based on intensity (fluorometry) (Idexx, 2015). The layers act as filters to try to minimise interference caused by physiological abnormalities but this method remains more susceptible to the effects of interferents than the indirect ISE method (Bernardini et al., 2009, Hubl et al., 1994).

1.6.2.2 IDEXX VetStat®

The IDEXX VetStat® Electrolyte and Blood Gas analyser (IDEXX Laboratories, Inc., Westbrook, Maine, U.S.A) is another commonly used POC analyser. It measures optical fluorescence (OF) from a blood sample analysed in a disposable single-use cassette via sensors called optodes. The electrolyte channels are calibrated with a buffer solution contained in the cassette. During measurement, light is passed through optical filters so that photons of a specific energy are transmitted to the sensors, causing them to emit fluorescence specific to each electrolytes concentration within the sample (Idexx, 2010).

1.7 Method Comparison Studies

The American Society of Veterinary Clinical Pathologists (ASVCP) publishes guidelines for performing method comparison studies and which statistical tests should be best employed; the latest version of these was published after data collection for this study had been completed but were broadly adhered to (Arnold et al., 2019). They recommend calculating the correlation coefficient (r) to help determine the appropriate statistical tests but that this is not acceptable as a measure of agreement. For data with a narrow range, such as electrolytes, if r is >0.975 then standard linear regression statistics can be used to estimate the bias at medical decision concentrations; however, for data that vary over a wide range, regression statistics are typically used. If r <0.975, it may be improved by collecting more data points or decreasing the variance by doing replicate measurements. Paired t-test statistics can be used to estimate the bias as the difference between the means of the results by the two methods, however, they are not applicable in the presence of proportional error. In this situation Passing-Bablok or Deming regression analysis should be used. Additionally, subdivision of results into groups (below, within, or above the reference interval) may provide additional insights for means in ranges that are clinically significant.

Bland-Altman plots should be created by plotting the difference between the two tests on the y-axis, and the mean (or median) of both methods on the x-axis. The line of difference identifies the mean bias. For tests with absent or negligible bias, results are scattered closely around the line of zero difference, with approximately half above and half below as well as a narrow range of individual differences.

1.8 Aims of this study

The aims of this study were:

1) to compare two commonly used point-of-care electrolyte analysers (IDEXX Catalyst™ Dx and VetStat®) accuracy against a reference laboratory's results (ISE) in blood samples from dogs with diagnosed primary hypoadrenocorticism.

2) to test the precision of these analysers through reproducibility studies of the results that were obtained and to assess the bias between analysers.

2 Materials &Methods

2.1 Hypothesis

The primary null hypothesis of this study was that the IDEXX Catalyst™ Dx and IDEXX VetStat® analysers would have good precision over a wide range for sodium, potassium and chloride concentrations. Secondary null hypotheses were that they would have high correlations and good agreement with results from the reference indirect ISE method and that any identified bias between the IDEXX Catalyst™ Dx or IDEXX VetStat® and the reference indirect ISE would not impact on clinical decisions.

2.2 Study Design

2.2.1 Experimental Design

The experiment was designed as a prospective single-site field study performed in the UK at the University of Glasgow, Small Animal Hospital between October 2015 and April 2019. Samples were initially collected from a concurrent two group, randomised, non-masked, non-inferiority cross-over study comparing two different treatments for the chronic management of hypoadrenocorticism in dogs (DOCP and fludrocortisone) and are the subject of another MVM thesis (Spence, 2020). After the non-inferiority study had concluded in January 2017, additional data was gathered, and further dogs recruited to increase the number of test samples available to conduct a method comparison study which forms the basis of this MVM thesis.

2.2.2 Sample Size Calculation

Sample size calculations were not performed for this study however were performed for the initial cross-over study comparing two different treatments. The ASVCP recommends that method comparison studies use a minimum of 40 independent patient samples tested by both methods (Jensen and Kjelgaard-Hansen, 2006). Human laboratory medicine recommendations state that imprecision and bias are assessed based on repeated measurement of at least 20 samples however this recommendation has been modified to 5 replications for veterinary POC analysers (Arnold et al., 2019).

2.2.3 Recording Data and Study Monitoring

Data was recorded at the time of consultation onto paper trial documents, which were labelled with the animals' hospital number, name and owner surname and visit number. Paperwork was stored securely between visits and at the end of the trial was manually entered into an electronic database. The paper copies were also digitised for review in future if needed. The trial monitor reviewed the electronic database to ensure accuracy. Owners also kept paper records at home of the medications they had administered and their observations of the dogs.

2.3 Case Selection

2.3.1 Animal Selection

Dogs were actively recruited from surrounding private first opinion small animal veterinary practices. There was no restriction on breed, gender or neutering status. For the cross-over study, dogs had to be over 5kg in weight due to the volume of blood being collected. This restriction was lifted for the rest of the study as blood was only collected for monitoring purposes.

2.3.2 Animal Identification

On presentation at the hospital, each animal was given a six-digit hospital number which was used along with the patients' name and owner surname, to identify all samples and study data.

2.3.3 Eligibility and Inclusion Criteria

Dogs were required to be privately owned and to have been diagnosed with hypoadrenocorticism using an ACTH stimulation test performed at an external reference laboratory, with pre and post ACTH cortisol levels <55 nmol/L. For the cross over study, they had to have been diagnosed at least 60 days previously and have been stable (as assessed by sodium and potassium concentrations within their references ranges at the first visit) for at least 30 days on a twice daily dose of fludrocortisone with or without supplemental prednisolone. This restriction was removed for the remainder of this study to be able to include all dogs with hypoadrenocorticism, from newly diagnosed to long term stable cases to ensure that the study was as representative as possible of primary care practice.

Animals with concurrent diabetes mellitus, hypothyroidism or urinary incontinence were not excluded from the study providing those individuals had only one of these concurrent conditions, and the condition was stable with treatment.

2.3.4 Exclusion Criteria

Dogs were excluded if they were uncooperative with study procedures (or became so during the study), if they were pregnant, lactating or had renal insufficiency (diagnosed by increased blood urea, creatinine and persistently isosthenuric urine despite intravenous fluid therapy). Additionally, they were excluded if they had clinical signs of primary hepatic failure (e.g. ascites, severe hypoalbuminemia etc), had congestive heart failure, were receiving immunosuppressive therapy or if they were receiving systemic steroid therapy other than prednisolone (for hypoadrenocorticism) or oestriol (for the treatment of urinary incontinence).

2.3.5 Ethical Approval

This study received University ethical approval (REF03a/17) and the non-inferiority trial was conducted under an Animal Test Certificate number ATC 10434/0002, granted to Dechra Limited on 7th July 2015 by the Veterinary Medicines Directorate of the UK. Subsequent enrolment of additional dogs with hypoadrenocorticism was performed under the Veterinary Surgeons Act 1966 with residual blood samples being used following routine monitoring.

2.3.6 Informed Consent

Informed consent, including a discussion of the risks of changing medication and the need for increased vigilance and monitoring during this time, was obtained from all owners before their animals were examined at the first visit. This was recorded via a signed consent form.

2.4 Treatment

2.4.1 Fludrocortisone

Fludrocortisone acetate (Florinef, 0.1mg tablets, Bristol-Meyers Squibb Pharmaceuticals Ltd) was the designated control product (CP) in the non-inferiority trial and is used, off-license, for the long-term treatment of canine hypoadrenocorticism. Due to availability problems during the trial, the CP was supplemented with generic Fludrocortisone acetate (Aspen Pharmacare Ltd) in some cases.

2.4.2 Desoxycortone pivalate

DOCP (Zycortal®, 25 mg/ml, Dechra Ltd) was the designated investigational product (IVP) during the non-inferiority trial. It is a prolonged release formulation of DOCP in a microcrystalline suspension which had been recently authorised for the treatment of hypoadrenocorticism in dogs when the trial was started. DOCP has a licensed starting dose of 2.2mg/kg to be administered subcutaneously every 28 days (European Medicines Agency, 2019).

2.4.3 Dose Adjustments

Owners were strictly prohibited from making dose adjustments at home, even in the presence of drug-related side effects. All dose adjustments were made by either a Diplomate or Resident of the European College of Veterinary Internal Medicine – Companion Animal. The prednisolone dose was adjusted based on the presence of clinical signs of glucocorticoid excess (i.e. polyuria, polydipsia, polyphagia, excessive panting, coat changes) or deficiency (i.e. vomiting, diarrhoea, weakness). The fludrocortisone and DOCP doses were adjusted based on electrolyte concentrations, with hyperkalaemia or hyponatremia prompting a dose increase and hypokalaemia or hypernatremia resulting in a dose reduction.

2.4.4 Concurrent Medications

Dog's receiving concurrent medications for the management of other conditions were not excluded from the study unless they were drugs which could interfere with the analysis of electrolytes and management of hypoadrenocorticism. This included diuretics and any systemic steroid medications other than those prescribed during the study.

2.5 Electrolyte Monitoring

2.5.1 Blood Sampling

Each dog was blood sampled at baseline and at each subsequent visit via jugular venepuncture. Samples were collected by the attending Veterinary Surgeon or a Registered Veterinary Nurse under their direction. The blood sample was then immediately transferred into a 1.3 ml blood collection tube containing lithium-heparin.

2.5.2 Point-of-Care Electrolyte Measurement

Electrolyte analysis was performed on a POC analyser within 10 minutes of blood sampling to allow for any changes in treatment to be made at the time of the consultation. Samples were preferentially run on the Catalyst analyser but when test clips were not available, the VetStat was used. Occasionally, at the investigator's discretion, tests were performed on both POC analysers.

Samples run on the Catalyst were centrifuged at 9000 revolutions per minute (RPM) for 3 minutes (5433 g) and 300 µL aliquots of heparinised plasma were analysed. The VetStat analyser instead used a 200 µL aliquot of heparinised whole blood drawn into a 1 ml syringe.

Testing materials were stored and handled as recommended by the manufacturer. Catalyst analyser test clips (Lyte 4 CLIP, IDEXX Laboratories, Westbrook, USA) were kept at -20°C until immediately prior to testing. The VetStat test cassettes (Electrolyte 8 Plus, IDEXX Laboratories, Westbrook, USA) were kept at room temperature between 4°C and 30°C.

Both analysers were installed to the manufacturer's specifications and serviced as recommended with daily and monthly quality control checks using quality control products supplied by the manufacturer (IDEXX Laboratories, 2019, IDEXX Laboratories, 2010). Results were shared anonymously with IDEXX's SmartService™ allowing for automatic software updates and remote calibration of the analysers.

2.5.3 Laboratory Electrolyte Measurement

A paired sample was sent to the on-site veterinary reference laboratory (Veterinary Diagnostic Services, University of Glasgow). These samples were refrigerated within 4 hours of collection and stored for a maximum of two days before analysis although the vast majority were tested the same day representing normal clinical practice in the hospital. The reference laboratory analyser utilised an indirect ISE methodology using 40 µL of heparinised plasma diluted 1:10 (Olympus AU640 or Siemens Dimension® Xpand Plus). The analyser was maintained according to the manufacturers' specifications and recommendations. The ISE component underwent calibration every four hours with internal quality controls performed daily using two levels. The reference laboratory also participates in the Bio-Rad International Clinical Chemistry Monthly Quality Assurance Scheme which includes electrolyte analysis.

2.6 Statistical Analysis

All data was transferred from paper records onto a digital spreadsheet (Microsoft Excel, USA). Statistical analysis was performed using commercially available statistical software (Analyse-It Software Ltd, Version 5.40, Leeds, UK and GraphPad Prism 8, San Diego, USA). For all analysis, P values <0.05 were considered statistically significant.

Individual electrolytes were analysed separately. For analysis of the Na^+: K^+ ratio, the reference laboratories reference interval of 27 to 40 was investigated first. A narrower Na^+: K^+ ratio range of 27 to 32 was also examined as this is defined as the "ideal range" in the public assessment report for the formulation of DOCP (Zycortal®, Dechra Ltd.) used in this study and licensed in Europe (European Medicines Agency, 2019). This narrower range is also the basis for dose adjustments of DOCP according to the data sheet.

2.6.1 Data Analysis

2.6.1.1 Comparison Plots

The data was evaluated for outliers and if found these were double checked to ensure that they had been transcribed correctly and analysed appropriately. No replicate measures were performed of these more extreme outliers due to sample availability. The reference laboratory routinely repeated analysis of samples if they were found to be outside the reference interval to confirm the result.

2.6.1.2 Normality Testing

The data was assessed for normality by Shapiro-Wilk tests and by visual inspection of graphical plots. As none of the data were normally distributed, non-parametric statistical tests were used for all comparisons.

2.6.1.3 Spearman's Correlation

The correlation between methods was measured using Spearman's rank correlation coefficient (r) solely to ensure that a linear relationship was present. It was expected that there would be a strong correlation between analysers as they were measuring the same analytes, and this was found however it was <0.975 for all analytes.

2.6.1.4 Bland-Altman Analysis

The agreement between methods was assessed by calculating the bias and displayed using Bland-Altman plots for each variable. As these differences were not normally distributed, 95% limits of agreement (LOA) were derived based on the 2.5th and 97.5th percentiles and displayed around the median (Altman and Bland, 1983, Bland and Altman, 1986, Bland and Altman, 1999, Ludbrook, 2010). Agreement was considered good when the LOA were within the ASVCP recommended allowable total error (TE_A) (Harr et al., 2013). For sodium and chloride this is ± 5% of the target value and for potassium it is ± 5% or ±10% at low concentrations. The clinical laboratory improvement amendment (CLIA) guidelines suggest a TE_A of ± 4.0mmol/L for sodium and ± 0.5mmol/L for potassium so both values were considered. No CLIA guidelines are defined for chloride (Harr et al., 2013).

2.6.1.5 Cohen's Kappa

To further assess the agreement between the IDEXX Catalyst and IDEXX VetStat and the reference ISE method, Cohen's kappa coefficient (κ) statistics were calculated. Results were collected into three categories based on if they fell below, within or above the reference interval for each analyser and then compared to the ISE analyser. The agreement was considered poor if $\kappa < 0.2$, fair if $\kappa = 0.21$ to 0.40, moderate if $\kappa = 0.41$ to 0.60, good if $\kappa = 0.61$ to 0.80 and very good if $\kappa > 0.81$ (Landis and Koch, 1977).

2.6.1.6 Precision Analysis

The inter-assay imprecisions of both the IDEXX Catalyst and IDEXX VetStat analysers were assessed by repeated analysis of three samples run five times consecutively on each analyser. Different levels of sodium, potassium and chloride were studied. This allowed determination of the coefficient of variation (CV), bias and intra-assay observed total error ($TE_{(Obs)}$) for each analyte on both analysers. The $TE_{(Obs)}$ was defined as Bias (%) + 2CV. The $TE_{(Obs)}$ was then compared to the TE_A of each analyte to determine if it was acceptable according to the ASVCP and CLIA guidelines i.e. if $TE_{(Obs)} < TE_A$. (Flatland et al., 2014, Arnold et al., 2019).

2.6.1.7 Passing-Bablok Regression Analysis

All analytes showed linear correlation however their r was <0.975 so Passing-Bablok regression analysis was performed to further investigate analyser bias. With Passing-Bablok regression, if the Y intercept differed significantly from 0 it was considered constant bias was present. Similarly, if the slope did not include 1 it was thought that there was proportional bias (Bablok and Passing, 1985, Jensen and Kjelgaard-Hansen, 2006).

2.6.1.8 Linear Regression Analysis

Linear regression was performed to evaluate the effects of individual patients, age, sex, neuter status, treatment (Fludrocortisone vs DOCP) and sample number. Other haematological and biochemical variables were not routinely recorded, other than at the start and end of each phase of the non-inferiority trial, so could not be analysed. Potentially significant variables ($P<0.2$) were carried forward into a generalised mixed linear model with significance set as $P<0.05$.

2.6.1.9 Sensitivity and Specificity

For both the IDEXX Catalyst™ Dx and IDEXX VetStat® analysers, the sensitivity and specificity were calculated for detecting sodium, potassium and chloride outside their reference interval relative to the ISE reference method. This assumed that the ISE method was 100% sensitive and specific. Receiver-operator characteristic (ROC) curve analysis was also performed to allow comparison of area under the curves (AUC) using the Delong method.

3 Results

3.1 Animals

3.1.1 Patients Enrolled

Forty-eight dogs diagnosed with hypoadrenocorticism and treated at the Small Animal Hospital were enrolled into a prospective cross-sectional study between October 2015 and April 2019. Thirty-three dogs were enrolled into the initial non-inferiority study (although two of these were later excluded) and a further 17 were subsequently recruited.

3.1.2 Signalment

The included dogs ranged in age from 0.5 to 13 years (mean 6.7 years, median 6 years). A range of breeds were represented including: Crossbreed (n=8), Standard Poodle (n=5), Cocker Spaniel (n=5), Labradoodle (n=5), Springer Spaniel (n=3), Labrador (n=3), West Highland White Terrier (n=2), Tibetan Terrier (n=2), Bearded Collie (n=2) and one each of Lhasa Apso, Jack Russel Terrier, Great Dane, Tibetan Mastiff, Toy Poodle, German Shorthaired Pointer, Utonagan, Husky, Cavalier King Charles Spaniel, Miniature Pinscher, Yorkshire Terrier, German Shepard Dog and Border Collie. They weighed between 2.05 kg and 69 kg (mean 22.3 kg, median 20.8 kg). Twenty-four of the dogs were male and 24 were female. Of these, 19 of each were neutered and five were entire. The signalment of the enrolled dogs are listed in Table 1.

3.1.3 Pre-existing Conditions

One patient was treated with glucocorticoid (dexamethasone) containing eye drops (Maxitrol, Alcon) for 13 days before its ACTH stimulation test. Two days prior to the test, prednisolone acetate drops were introduced (Pred-Forte, Allergen Ltd.) when it was diagnosed with chronic superficial keratitis (pannus). These drops were continued throughout the study period. Another patient was urinary incontinent and treated with oral oestradiol (Incurin, MSD Animal Health) throughout the study. Finally, another dog had long-standing recurrent Malassezia otitis externa and was recently treated with oral ketoconazole (Sporanox, Jassen-Cilag Ltd.) and amoxicillin/clavulanic acid (Synulox, Zoetis).

Table 1 – Signalment of Dogs Enrolled

Dog No.	Age (Yrs)	Sex	Breed	Weight (Kg)	Pre-ACTH Cortisol (nmol/L)	Post-ACTH Cortisol (nmol/L)
1	11	FN	Crossbreed	11.1	<7	<7
2	0.5	FE	Labradoodle	14.8	<10.5	<10.5
3	10	FN	JRT	7.6	<14	<14
4	4	MN	Great Dane	69	14	72
5	4	FN	Cocker Spaniel	12.8	27	42
6	7	FN	Crossbreed	49.4	<7	<7
7	12	MN	West Highland White Terrier	10.6	18	14
8	4	FN	Labradoodle	40	<7	<7
9	9	MN	Crossbreed	27.5	<6.9	<6.9
10	9	MN	Bearded collie	21.5	<6.9	<6.9
11	7	FN	Tibetan Mastiff	30.8	30	35
12	5	FN	West Highland White Terrier	6.3	<6.5	<6.5
13	7	MN	Standard Poodle	25	<10.5	<10.5
14	4	MN	Standard Poodle	33.1	<7	<7
15	7	MN	Labrador	33.5	<7	<7
16	8	FN	Springer Spaniel	18.9	44	45
17	11	MN	Cocker Spaniel	16.1	13	9
18	10	MN	Cocker Spaniel	15.6	19.5	22.4
19	9	MN	Toy Poodle	7.6	<5.6	<5.6
20	3	MN	German Short Haired Pointer	31.3	27.6	27.6
21	9	MN	Standard Poodle	33.6	<7	<7
22	6	FE	Border Collie	18.5	34	38
23	5	MN	Tibetan Terrier	9.3	<10	<10
24	2	MN	Standard Poodle	24.7	<27.6	<27.6
25	5	FE	Utanagon	22.9	13.5	<10.5
26	3	MN	Bearded collie	20.3	<10	<10
27	1	FE	Labrador	32.8	7.2	7.92
28	7	ME	Springer Spaniel	29.4	<14	<14
29	4	FE	Crossbreed (Bearded Collie)	14.3	<14	<14
30	6	FN	Cavalier King Charles Spaniel	11.3	<7	<7
31	3	MN	Husky	40.2	<6.9	<6.9
32	9	FN	Labrador	42.1	19	21
33	3	FN	Miniature Pinscher	5.9	48	22
34	7	FN	Cocker Spaniel	9.7	21	23
35	6	ME	Labradoodle	28.8	8	<6.9
36	12	FN	Crossbreed	17.8	<6.9	<6.9
37	11	MN	Tibetan Terrier	20.8	<6.9	<6.9
38	11	FN	Crossbreed	30.9	<7	<7

39	5.5	ME	Crossbreed	5.7	<7	<7
40	8	ME	Springer Spaniel	22	<6.9	<6.9
41	2	ME	Yorkshire Terrier	2.05	<6.9	<6.9
42	4	MN	Labradoodle	19.2	<27.6	<27.6
43	3	FN	Standard Poodle	26.1	<6.9	<6.9
44	5	FN	German Shepherd Dog	33	<6.9	<6.9
45	13	MN	Lhasa Apso	9.2	23	20
46	6	FN	Cocker Spaniel	13.9	<6.9	<6.9
47	10	FN	Labradoodle	25.1	<6.9	<6.9
48	13	FN	Crossbreed	17.8	<6.9	<6.9

Key: MN = Male Neutered, ME= Male Entire, FN = Female Neutered, FE = Female Entire

3.1.4 Concurrent Medications

Due to the duration of the study, several animals developed conditions requiring medications unrelated to their hypoadrenocorticism or its treatment by their primary care veterinary practices. Five dogs were prescribed antibiotics for various conditions; three received amoxicillin/clavulanic acid to treat superficial pyoderma and a fourth for a suspected urinary tract infection. The fifth dog was prescribed clindamycin for the management of colitis. Two dogs were given tramadol, one for the management of transient hind limb lameness and the other following a minor road traffic accident following transition from IV methadone. This dog was also given an increased dose of glucocorticoids initially following the accident with IV dexamethasone. One dog was prescribed clotrimazole following a flare up of *Malassezia* otitis externa. Another dog was started on phenylpropanolamine (Propalin, Vetoquinol) to manage urinary incontinence.

Two dogs required additional treatments possibly due to adverse effects of DOCP. One dog was hospitalised to treat lethargy, anorexia, hypokalaemia and hypotension with IV fluid therapy, potassium chloride, maropitant and spironolactone. Intravenous buprenorphine was also administered when it was suspected he had developed pancreatitis. Another dog was administered maropitant for suspected nausea following a single vomit, lip smacking and inappetence. None of these treatments were expected to affect the measurement of the dog's electrolytes.

3.1.5 Patients Excluded

Two dogs were excluded from the study after recruitment due to deviations from the enrolment criteria. One dog's diagnosis of hypoadrenocorticism had not been confirmed by an ACTH stimulation test and was based on compatible abnormal electrolyte concentrations and signalment (a young Bearded Collie). Another dog with persistent hypercholesterolaemia of 17.45 mmol/L (RI 2.0-7.0) was excluded as this may have effected electrolyte analysis and it was subsequently diagnosed with hypothyroidism during the study based on an undetectable thyroxine (T4) level and increased thyroid stimulation hormone (TSH) level of 3.54 ng/ml (RI 0 – 0.61). It was felt the dog was truly hypothyroid rather than simply having suppressed T4 due to exogenous glucocorticoid therapy (O'Neill et al., 2011, Torres et al., 1991, Daminet and Ferguson, 2003, Reusch et al., 2017). Treatment with levothyroxine was instituted and resulted in an improvement in the dog's demeanour, reduction in cholesterol and normalisation of T4.

A third dog failed to complete the non-inferiority trial following its sudden death one week after enrolment, but its samples were included in this study. Post-mortem examination was consistent with a haemoabdomen due to a ruptured haemangiosarcoma.

3.2 Protocol Deviations and Adverse Events

There were two main adverse events during the trial. As mentioned, one dog died of a ruptured splenic haemangiosarcoma which was deemed unconnected to the trial. Another dog developed suspected pancreatitis following administration of DOCP although it recovered completely and was able to complete the trial. All samples were included in the final analysis.

3.3 Study Results

3.3.1 Study Design

There was one significant change made to the study design during the trial which may have affected this study. This involved the reference laboratory changing their indirect ISE analyser from the Olympus AU640 to the Siemens Dimension Expand Plus. Both analysers were run in parallel for several weeks and found to produce comparable results without altering the normal reference interval for this method. The normal reference intervals for all three methods are listed in Table 2.

Table 2 – Reference intervals for the reference laboratory ISE method, IDEXX Catalyst™ Dx and IDEXX VetStat® analysers

Analyte	Reference ISE	IDEXX Catalyst	IDEXX VetStat
Na^+ (mmol/L)	136 – 159	144 – 160	144 – 160
K^+ (mmol/L)	3.4 – 5.8	3.5 – 5.8	3.5 – 5.8
Na^+: K^+	27 – 40	-	-
Cl^- (mmol/L)	95 – 115	109 – 122	109 – 122

3.3.2 Number of Samples Measured

A total of 401 paired samples were measured on one of the POC analysers and by the reference laboratory indirect ISE method; 329 on the IDEXX Catalyst™ Dx analyser and 72 on the IDEXX VetStat®. Only twelve samples were analysed on both POC analysers but were treated independently for statistical analysis as this was such a small proportion of the total.

3.3.3 Number of Samples per dog

Some dogs had only one blood sample tested whilst others had up to 18 samples included at various time points, measured between both analysers. There were 45 dogs tested on the IDEXX Catalyst™ Dx (median of 8 samples per dog, range 1 – 14 samples) and 29 dogs tested on the IDEXX VetStat® (median of 1 sample per dog, range 1 – 4 samples). A sub-analysis of the data, including only one sample per dog, found generally similar Spearman's correlation coefficients to those of the whole data set. This was much closer for the VetStat compared to the Catalyst. When there were differences, the correlation was generally worse other than the sodium measured on the Catalyst and chloride measured on both analysers (Catalyst: $Na^+ = 0.67$, $K^+ = 0.72$, Na^+: $K^+ = 0.79$, $Cl^- = 0.78$ and VetStat: $Na^+ = 0.71$, $K^+ = 0.89$, Na^+: $K^+ = 0.92$, $Cl^- = 0.68$).

3.3.4 Sample Test Ranges

The range over which samples were tested on both POC analysers and the reference indirect ISE method are listed below in Table 3.

Table 3 – Range over which samples were tested on the IDEXX Catalyst™ Dx, IDEXX VetStat® and reference ISE method.

Catalyst						
Analyte	No. of Samples	ISE Mean	ISE Range	Catalyst Mean	Catalyst Range	Spearman's Correlation
Na^+	329	146.9	121.1 – 159.4	152.1	123 –165	0.60
K^+	329	4.2	2.9 – 8.2	4.4	2.9 – 8.6	0.83
Na^+: K^+	329	35.9	15.6 – 51.5	35.9	15 – 54	0.86
Cl^-	329	110.5	87 – 129.9	113.3	92 – 130	0.64
VetStat						
Analyte	No. of Samples	ISE Mean	ISE Range	VetStat Mean	VetStat Range	Spearman's Correlation
Na^+	72	146.1	128.1 - 159.4	156.7	129 - 164	0.69
K^+	72	4.3	2.9 - 8.2	4.2	2.7 - 7	0.87
Na^+: K^+	72	35.5	15.6 - 51.3	38.6	18.4 - 53.7	0.89
Cl^-	72	109.6	93.1 - 118.1	116.4	102 - 123	0.67

3.3.5 Linear Regression

There was no significant association with individual dog, age, sex, treatment (DOCP and prednisolone vs fludrocortisone) or sample number identified. Very few parameters were significant at the $P < 0.2$ threshold and all became insignificant when carried into a general mixed linear model.

3.4 IDEXX Catalyst™

3.4.1 Bland-Altman Analysis

The IDEXX Catalyst™ Dx gave higher median results than the reference indirect ISE method for sodium and chloride concentrations but was much closer for potassium. For sodium, the median difference was unacceptably high at 5.0 mmol/L compared to the clinical target of 4.0 mmol/L. The LOA were also very wide (-6.0 to 12.3 mmol/L; -150% to 308%) and well outside this target. In contrast, the potassium concentration had a median difference of just 0.2 mmol/L achieving the CLIA clinical target difference of < 0.5 mmol/L. However, despite the much narrower LOA (-0.9 to 0.7 mmol/L; -180% to 140%) the range was unacceptable. Due to the smaller range over which potassium is measured and maintained by the body these differences may be clinically important. The median chloride concentration difference was acceptable at 2.5 mmol/L but the wide LOA (-2.5 to 9.6; -62.5% to 240%) were unacceptable if using the sodium CLIA target. This unreliability, especially of the sodium concentration caused a wide LOA in the Na^+: K^+ ratio results (Table 4, Figure 1).

3.4.2 Cohen's Kappa Agreement

The agreement for sodium and potassium were moderate at 0.51 and 0.46 respectively. Chloride was only fair at 0.33. The agreement of the Na^+: K^+ ratio was good at 0.61 which remained so at 0.63 even when the narrower range of 27 to 32 was applied (Table 5, Figure 2).

3.4.3 Passing-Bablok Regression Analysis

Sodium and chloride showed constant and proportional bias whereas potassium and the Na^+: K^+ ratio did not (Table 6, Figure 3). Compared to the reference ISE method the Catalyst tended to under-estimate at lower sodium concentrations and over-estimate at higher concentrations, whilst the reverse was true of chloride concentrations.

3.5 IDEXX VetStat®

3.5.1 Bland-Altman Analysis

The IDEXX VetStat® also gave higher median results than the reference ISE method for all analytes other than potassium (Table 4, Figure 4). The sodium analysis had an even worse median difference than the Catalyst of 11.5 mmol/L with wide LOA (-0.05 to 16.9 mmol/L; -1.25% to 423%), also failing to achieve the clinical target of 4.0 mmol/L. Similarly, chloride analysis was unacceptable with a median difference of 6.3 mmol/L and wide LOA (1.2 to 12.9 mmol/L; -30% to 323%) if using the sodium CLIA. Potassium analysis however performed better with an acceptable median difference of 0 mmol/L and narrower LOA (-1.4 to 0.6 mmol/L; -280 to 120%), although this still failed to meet the CLIA target of < 0.5 mmol/L difference. Given the poorer performance of the sodium analysis, the Na^+: K^+ difference was greater with wider LOA than the Catalyst.

3.5.2 Cohen's Kappa Agreement

The agreement for sodium was fair at 0.24, whilst is was poor for chloride at 0.14, However, it was very good for potassium at 0.82. The Na^+: K^+ ratio was moderate at 0.60 but when a narrower range of 27 to 32 was applied it fell to moderate at 0.48 (Table 5, Figure 5).

3.5.3 Passing-Bablok Regression Analysis

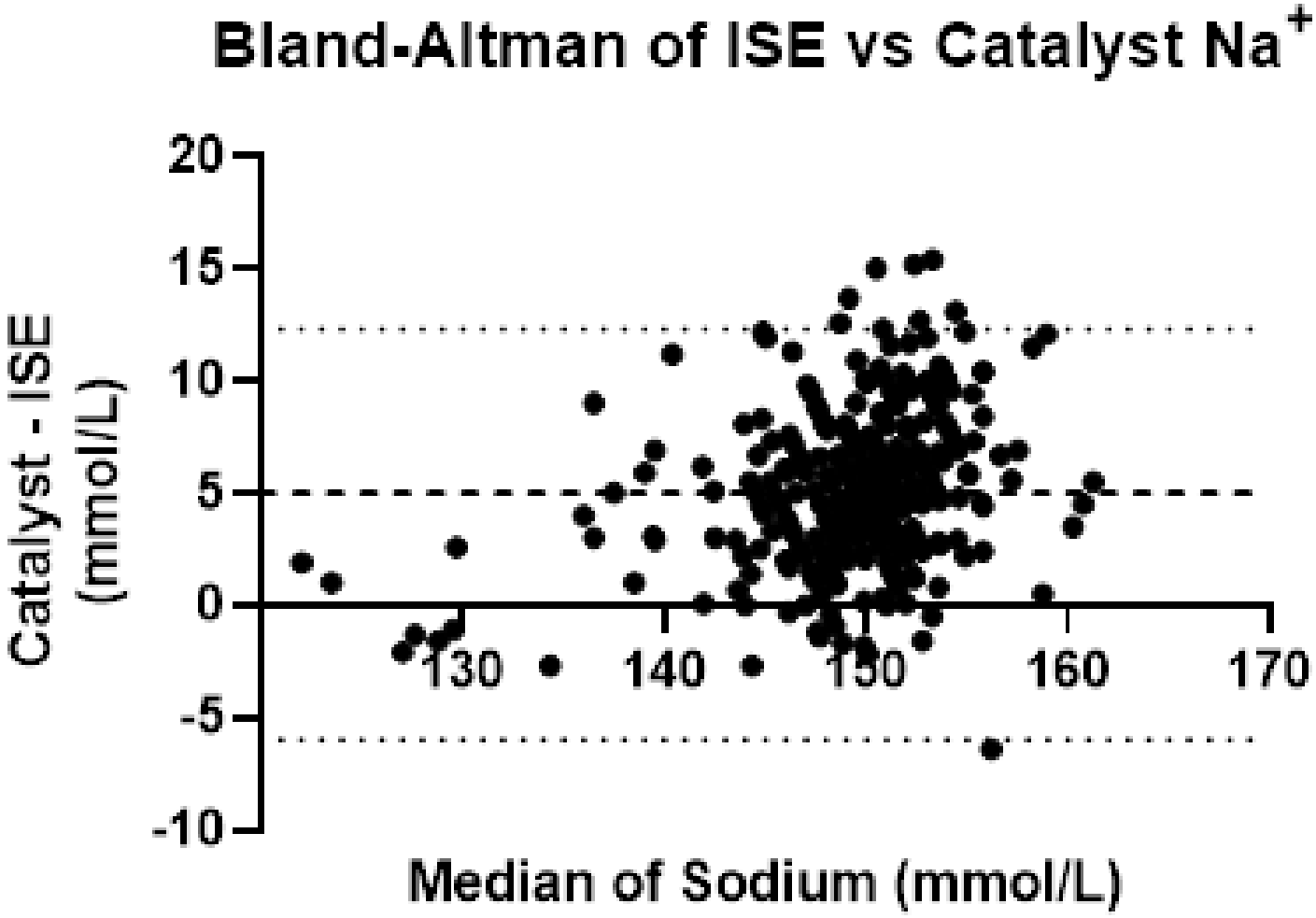
No bias was identified for potassium concentrations measured on the VetStat. A constant and proportional bias was identified for sodium and chloride analysis and proportional only for the Na^+: K^+ ratio (Table 6, Figure 6). Compared to the reference ISE method, the VetStat tended to have larger errors in chloride analysis at lower concentrations and smaller errors at higher concentrations whilst the opposite was true for sodium.

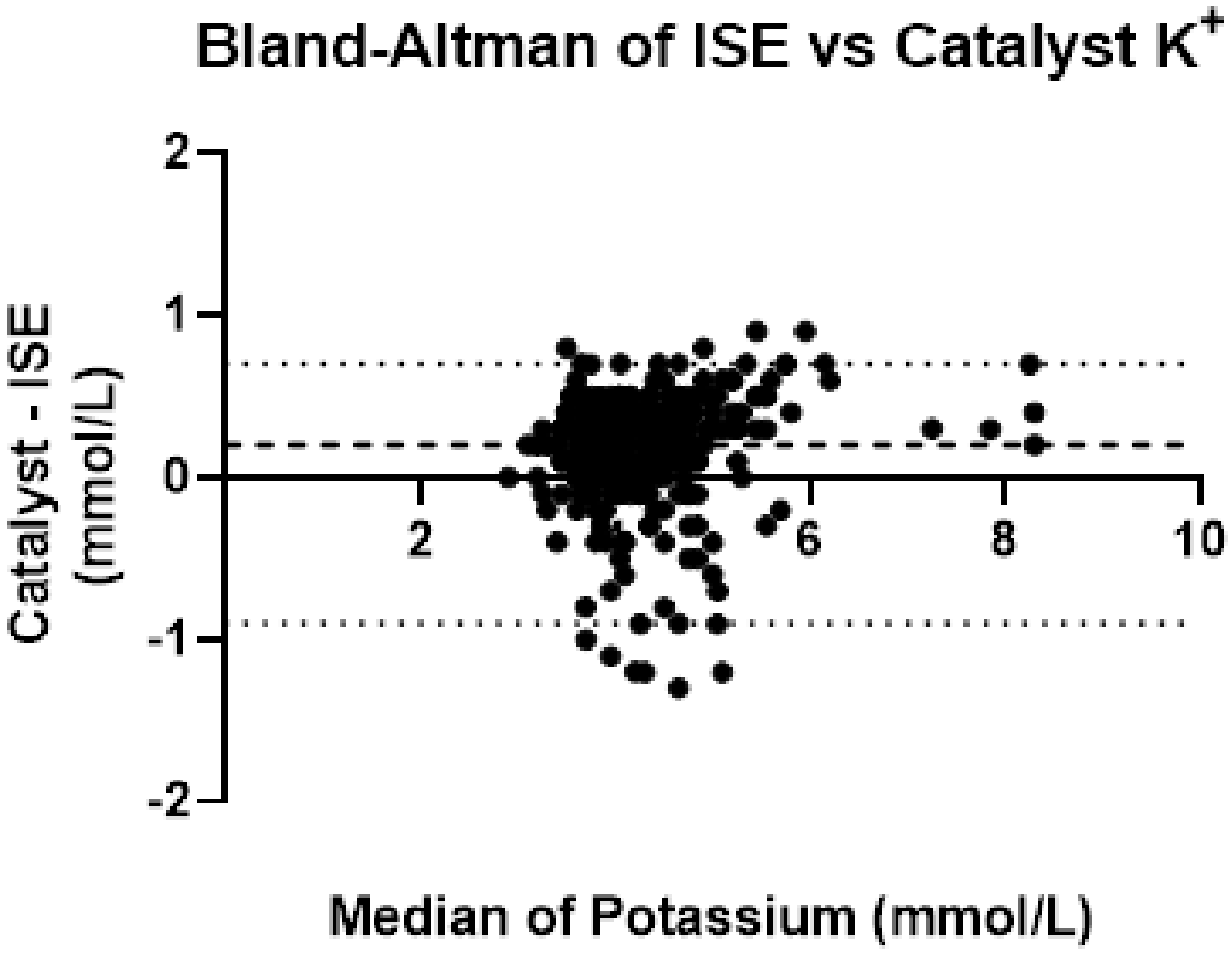
Table 4 – Summary of Bland-Altman analysis of the IDEXX Catalyst™ Dx and IDEXX VetStat® analysers compared to the reference ISE method.

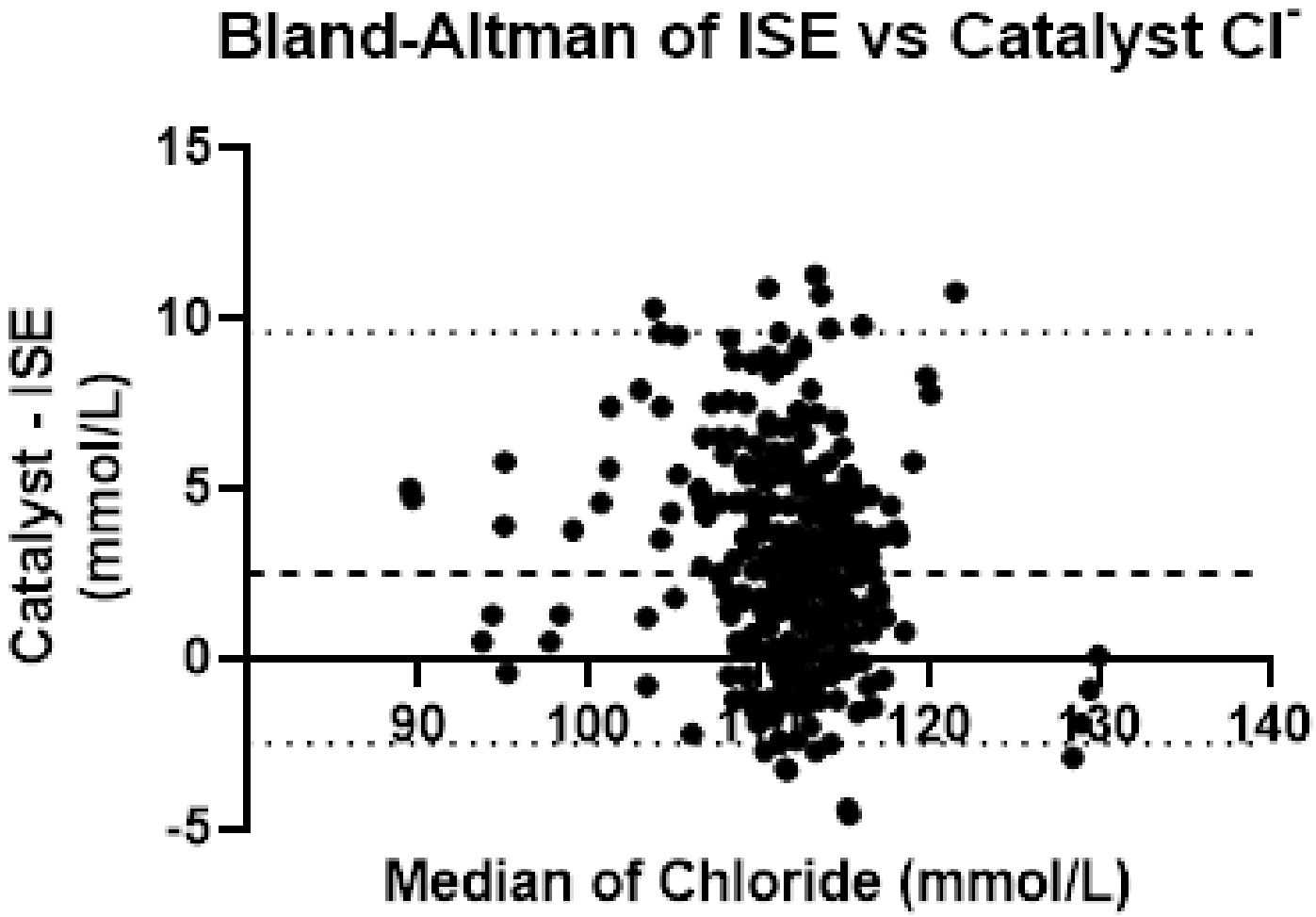
Catalyst				
Analyte	Median Difference + 95% CI	Lower LOA + 95% CI	Upper LOA + 95% CI	r coefficient
Na^+	5.0 (4.6 to 5.4)	-6.0 (-2.7 to -0.9)	12.3 (11.6 to 15.0)	0.81
K^+	0.2 (0.1 to 0.2)	-0.9 (-1.2 to -0.6)	0.7 (0.6 to 0.8)	0.90
Na^+: K^+	-0.4 (-0.6 to 0.0)	-4.6 (-5.7 to -3.9)	8.0 (6.0 to 9.5)	0.88
Cl^-	2.5 (2.0 to 3.0)	-2.5 (-3.3 to -1.9)	9.6 (8.8 to 10.8)	0.83
VetStat				
Analyte	Median Difference + 95% CI	Lower LOA	Upper LOA	r coefficient
Na^+	11.5 (10.6 to 12.1)	-0.05	16.9	0.84
K^+	0 (0 to 0)	-1.4	0.6	0.89
Na^+: K^+	2.6 (2.3 to 3.2)	-4.0	10.4	0.91
Cl^-	6.3 (5.7 to 6.7)	1.2	12.9	0.80

Key: CI = Confidence Interval, N.B. CI could not be calculated for the IDEXX VetStat

Figure 1 – Bland-Altman Plots for Sodium, Potassium, Chloride and Na^+: K^+ ratios on the IDEXX Catalyst™ Dx. The dotted lines represent the upper and lower limits of agreement (LOA) and the dashed line represents the median difference.




Bland-Altman of ISE vs Catalyst K+
Catalyst - ISE (mmol/L)
2
1
0
-1
-2
2
6
8
10
Median of Potassium (mmol/L)


Bland-Altman of ISE vs Catalyst Cl-
Catalyst - ISE (mmol/L)
15
10
5
0
-5
90
100
120
130
140
Median of Chloride (mmol/L)

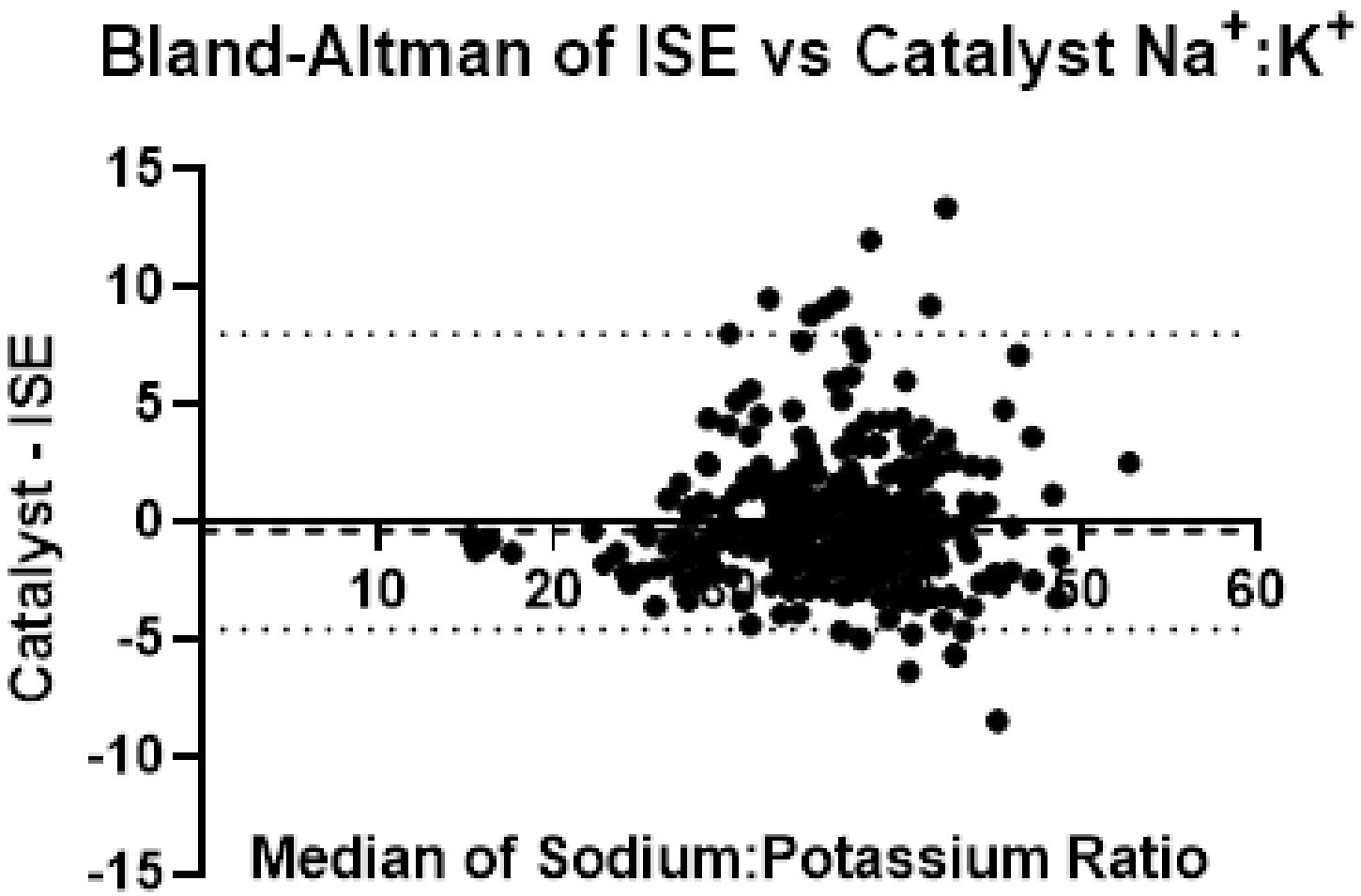

Bland-Altman of ISE vs Catalyst Na+:K+
Catalyst - ISE
15
10
5
0
-5
-10
-15
10
20
50
60
Median of Sodium:Potassium Ratio

Table 5 – Summary of Cohen's Kappa coefficient analysis of the IDEXX Catalyst ™ Dx and IDEXX VetStat ® analysers compared to the reference ISE method.

Analyte	Kappa Coefficient (κ)	95% Confidence Interval	Standard Error
Catalyst			
Na^+	0.51	0.33 to 0.69	0.09
K^+	0.46	0.29 to 0.63	0.09
Na^+: K^+ (27 – 40)	0.61	0.52 to 0.71	0.05
Na^+: K^+ (27 – 32)	0.63	0.54 to 0.72	0.05
Cl^-	0.33	0.19 to 0.47	0.07
VetStat			
Na^+	0.24	0.03 to 0.45	0.10
K^+	0.82	0.63 to 1.0	0.10
Na^+: K^+ (27 – 40)	0.60	0.42 to 0.78	0.09
Na^+: K^+ (27 – 32)	0.48	0.26 to 0.70	0.11
Cl-	0.14	-0.17 to 0.45	0.16

Figure 2 – Cohen's Kappa coefficient analysis of the IDEXX Catalyst™ Dx compared to the reference ISE method.

Frequencies

N	329

	Catalyst Na L/N/H			
ISE Na L/N/H	Low	Normal	High	Total
Low	12	1	0	13
	3.6%	0.3%	0.0%	4.0%
Normal	7	296	12	315
	2.1%	90.0%	3.6%	95.7%
High	0	1	0	1
	0.0%	0.3%	0.0%	0.3%
Total	19	298	12	329
	5.8%	90.6%	3.6%	

Agreement

Kappa	0.51
Wald 95% CI	0.33 to 0.69
SE	0.091

Frequencies

N	329

	Catalyst K L/N/H			
ISE K L/N/H	Low	Normal	High	Total
Low	7	14	0	21
	2.1%	4.3%	0.0%	6.4%
Normal	6	289	7	302
	1.8%	87.8%	2.1%	91.8%
High	0	0	6	6
	0.0%	0.0%	1.8%	1.8%
Total	13	303	13	329
	4.0%	92.1%	4.0%	

Agreement

Kappa	0.46
Wald 95% CI	0.29 to 0.63
SE	0.088

Frequencies

N	329

ISE Cl L/N/H	Catalyst Cl L/N/H Low	Normal	High	Total
Low	6	0	0	6
	1.8%	0.0%	0.0%	1.8%
Normal	19	269	0	288
	5.8%	81.8%	0.0%	87.5%
High	0	27	8	35
	0.0%	8.2%	2.4%	10.6%
Total	25	296	8	329
	7.6%	90.0%	2.4%	

Agreement

Kappa	0.33
Wald 95% CI	0.19 to 0.47
SE	0.073

Frequencies

N	329

ISE Na:K L/N/H	Catalyst Na:K L/N/H Low	Normal	High	Total
Low	13	4	0	17
	4.0%	1.2%	0.0%	5.2%
Normal	6	211	20	237
	1.8%	64.1%	6.1%	72.0%
High	0	24	51	75
	0.0%	7.3%	15.5%	22.8%
Total	19	239	71	329
	5.8%	72.6%	21.6%	

Agreement

Kappa	0.61
Wald 95% CI	0.52 to 0.71
SE	0.048

Frequencies

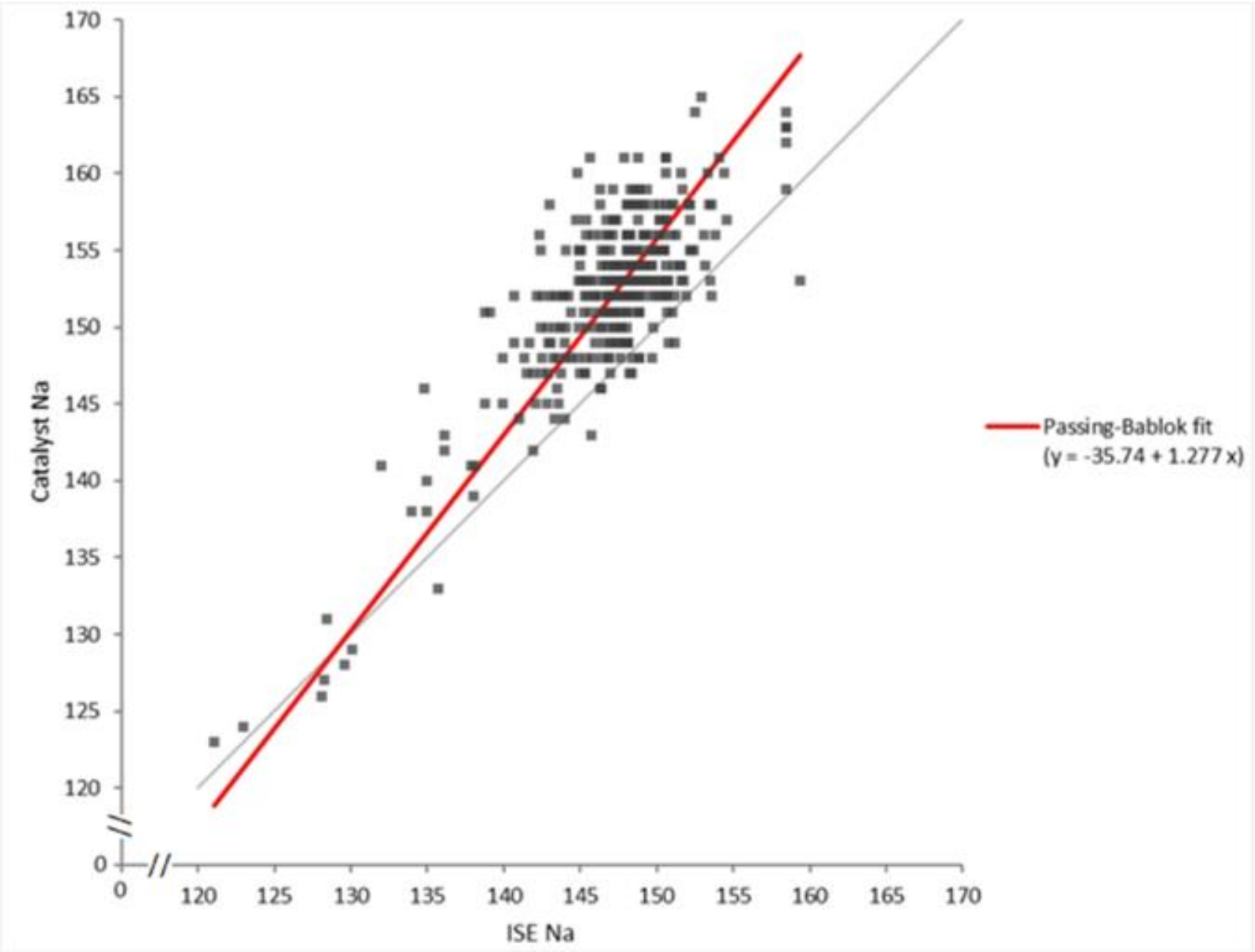
N	329

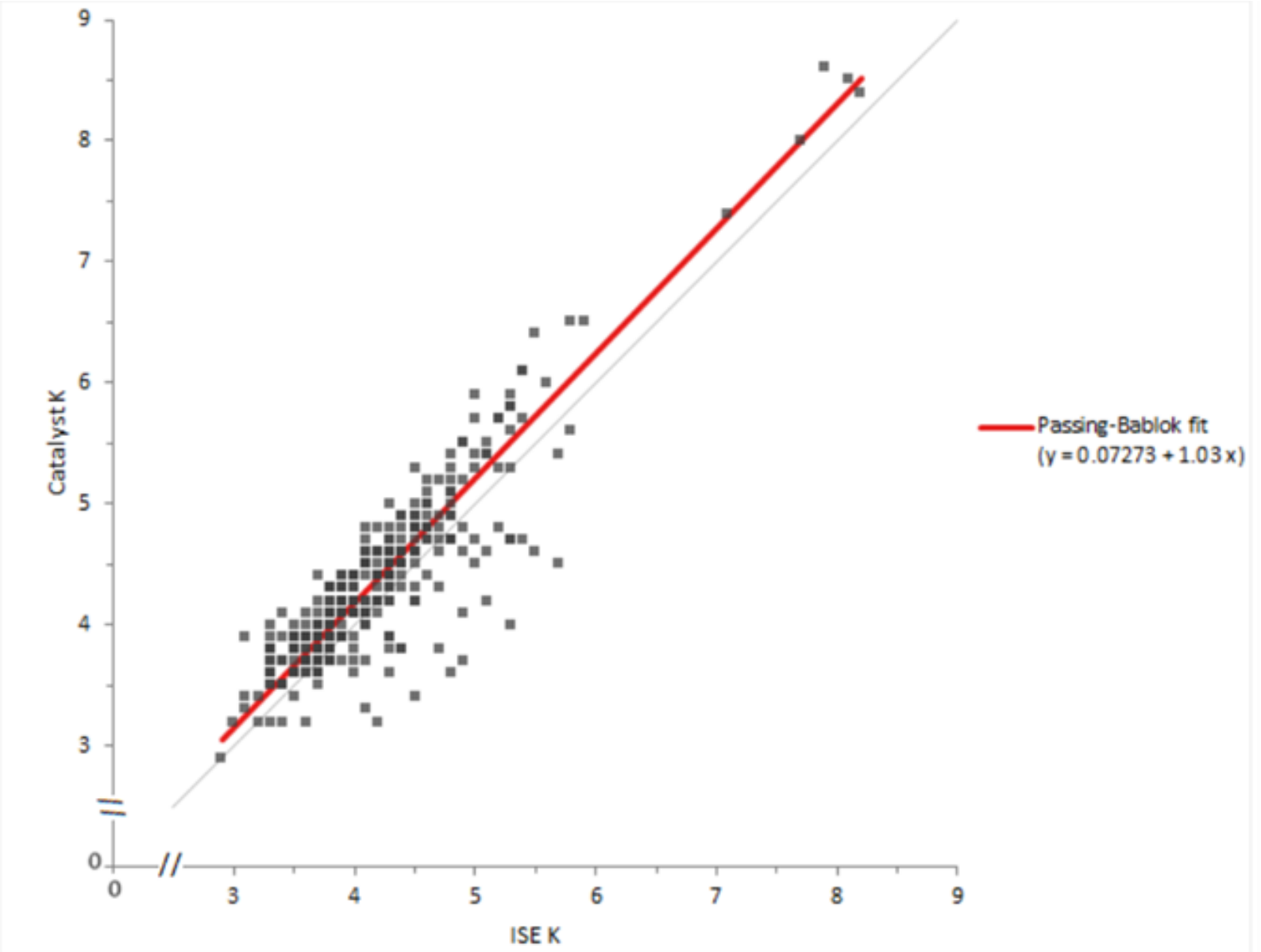
ISE Na:K 27-32 L/N/H	Catalyst Na:K 27-32 L/N/H Low	Normal	High	Total
Low	13	3	1	17
	4.0%	0.9%	0.3%	5.2%
Normal	6	38	18	62
	1.8%	11.6%	5.5%	18.8%
High	0	19	231	250
	0.0%	5.8%	70.2%	76.0%
Total	19	60	250	329
	5.8%	18.2%	76.0%	

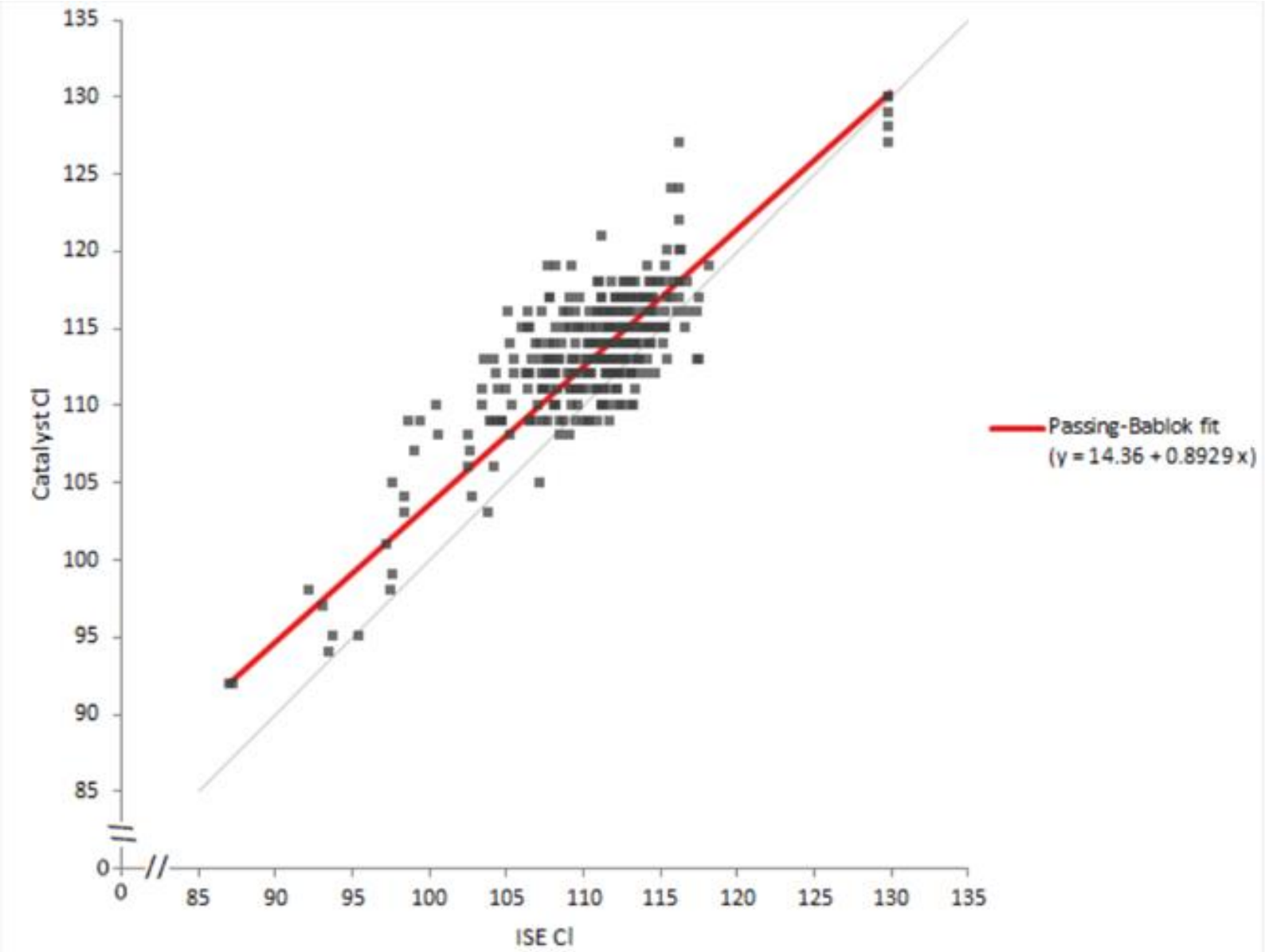
Agreement

Kappa	0.63
Wald 95% CI	0.54 to 0.72
SE	0.047

Figure 3 – Passing-Bablok regression analysis of the IDEXX Catalyst™ Dx compared to the reference ISE method.




Catalyst K
ISE K
Passing-Bablok fit
(y = 0.07273 + 1.03 x)


Catalyst Cl
ISE Cl
Passing-Bablok fit
(y = 14.36 + 0.8929 x)
135
130
125
120
115
110
105
100
95
90
85
0
0
85
90
95
100
105
110
115
120
125
130
135

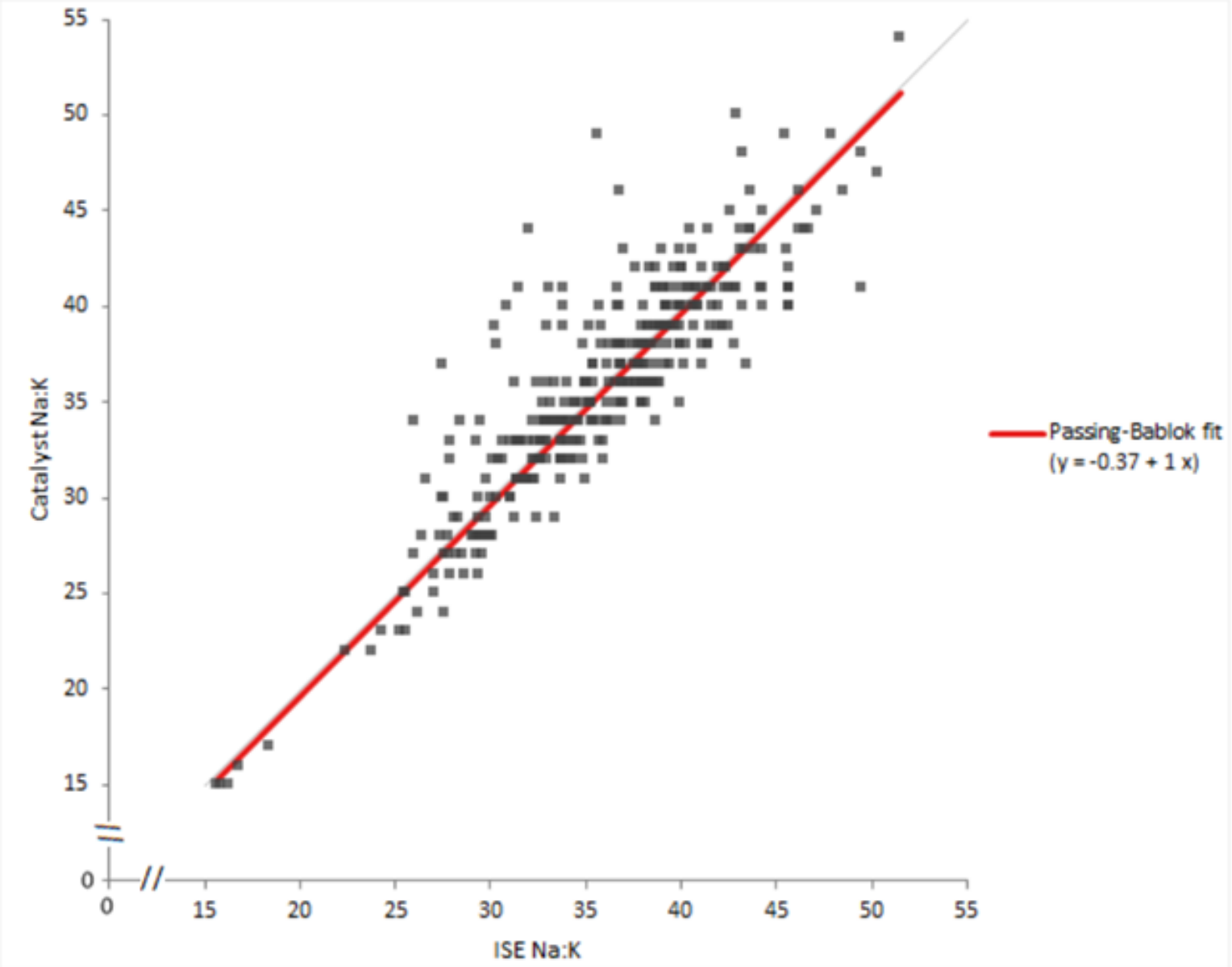
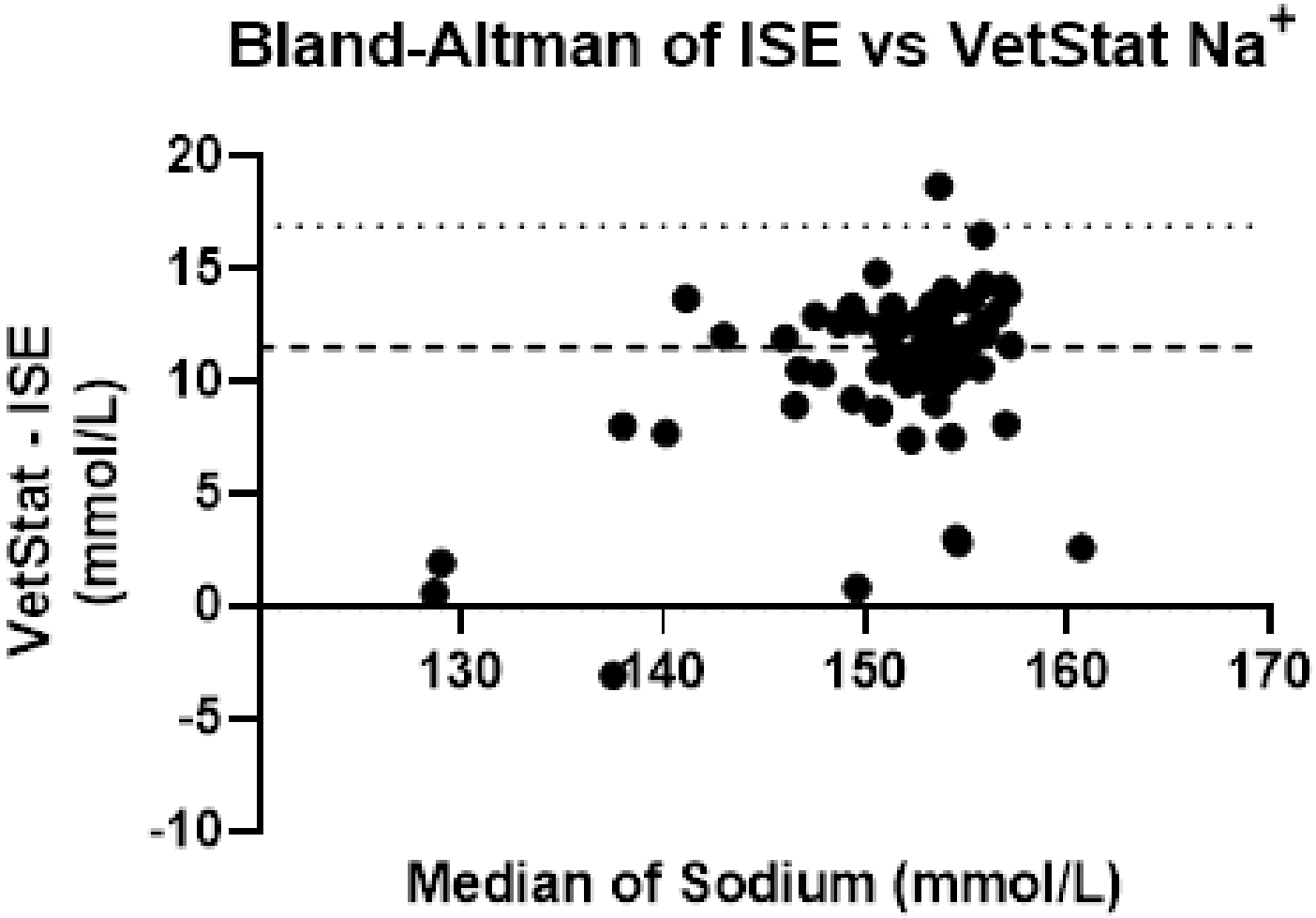
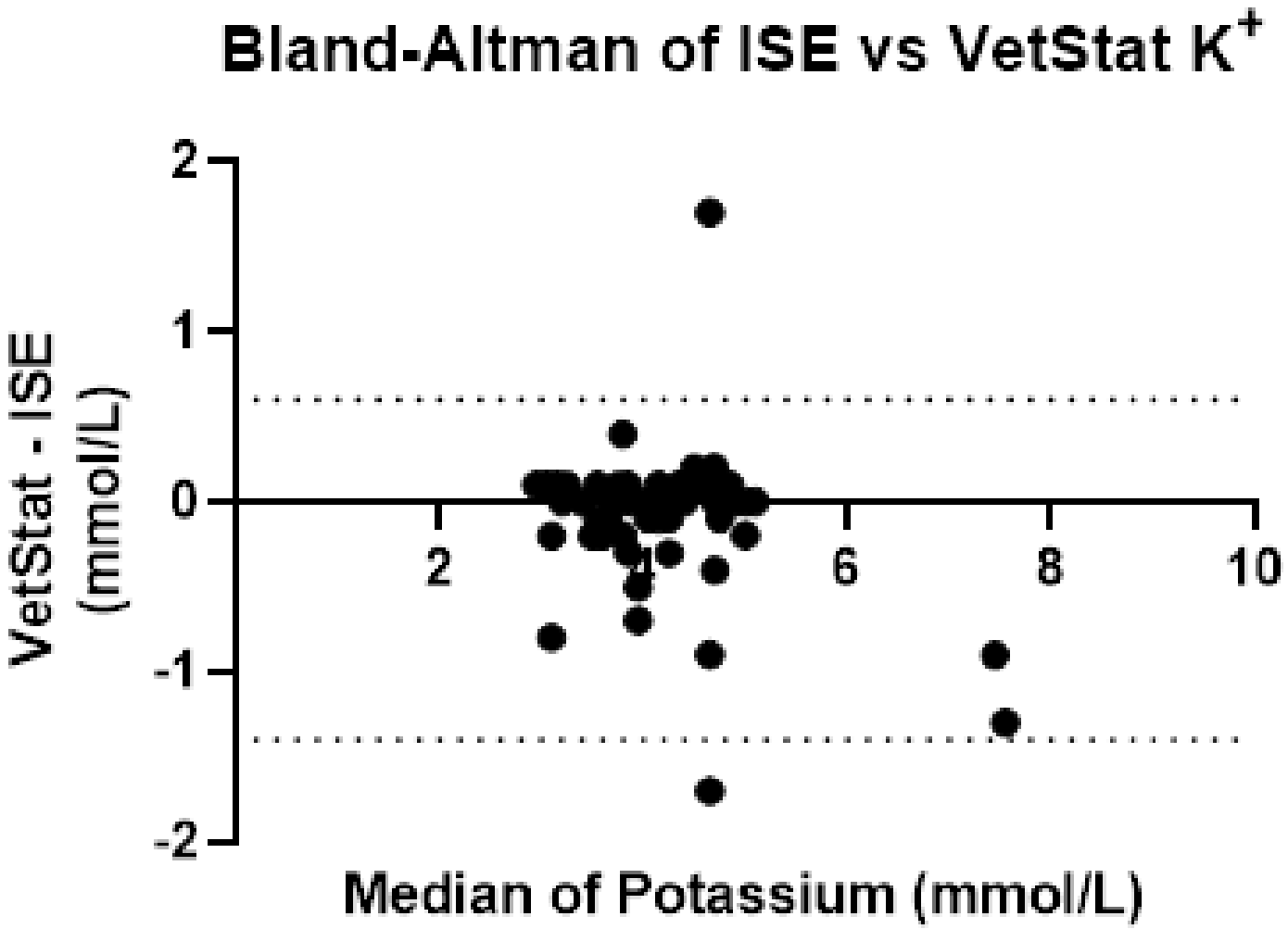
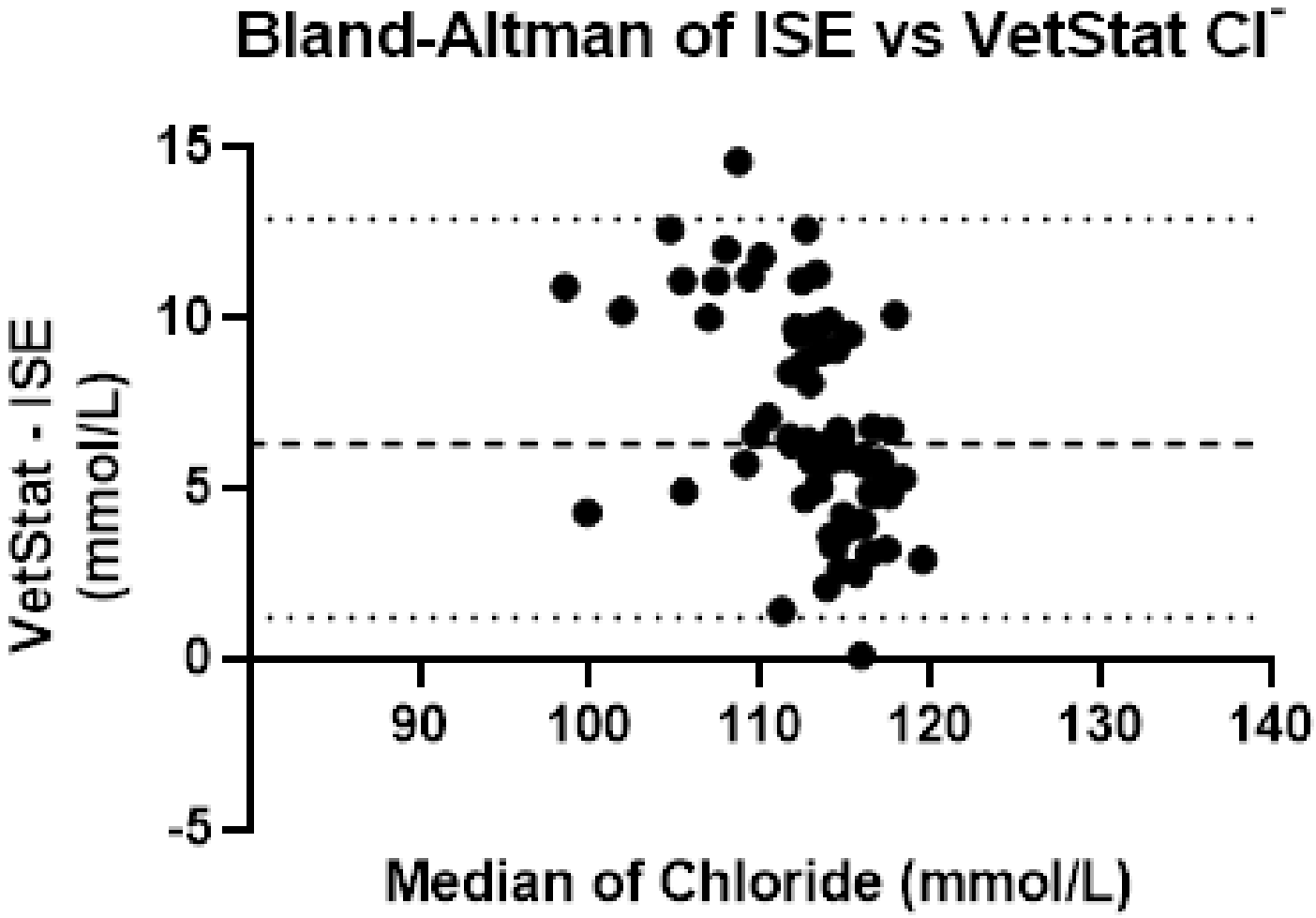

55
50
45
40
35
30
25
20
15
0
0
15
20
25
30
35
40
45
50
55
Catalyst Na:K
ISE Na:K
Passing-Bablok fit
(y = -0.37 + 1 x)

Figure 4 – Bland-Altman Plots for Sodium, Potassium, Chloride and Na^+: K^+ ratios on the IDEXX VetStat®. The dotted lines represent the upper and lower limits of agreement (LOA) and the dashed line represents the median difference.




Bland-Altman of ISE vs VetStat K+
VetStat - ISE (mmol/L)
2
1
0
-1
-2
2
6
8
10
Median of Potassium (mmol/L)


Bland-Altman of ISE vs VetStat Cl-
VetStat - ISE (mmol/L)
15
10
5
0
-5
90
100
110
120
130
140
Median of Chloride (mmol/L)

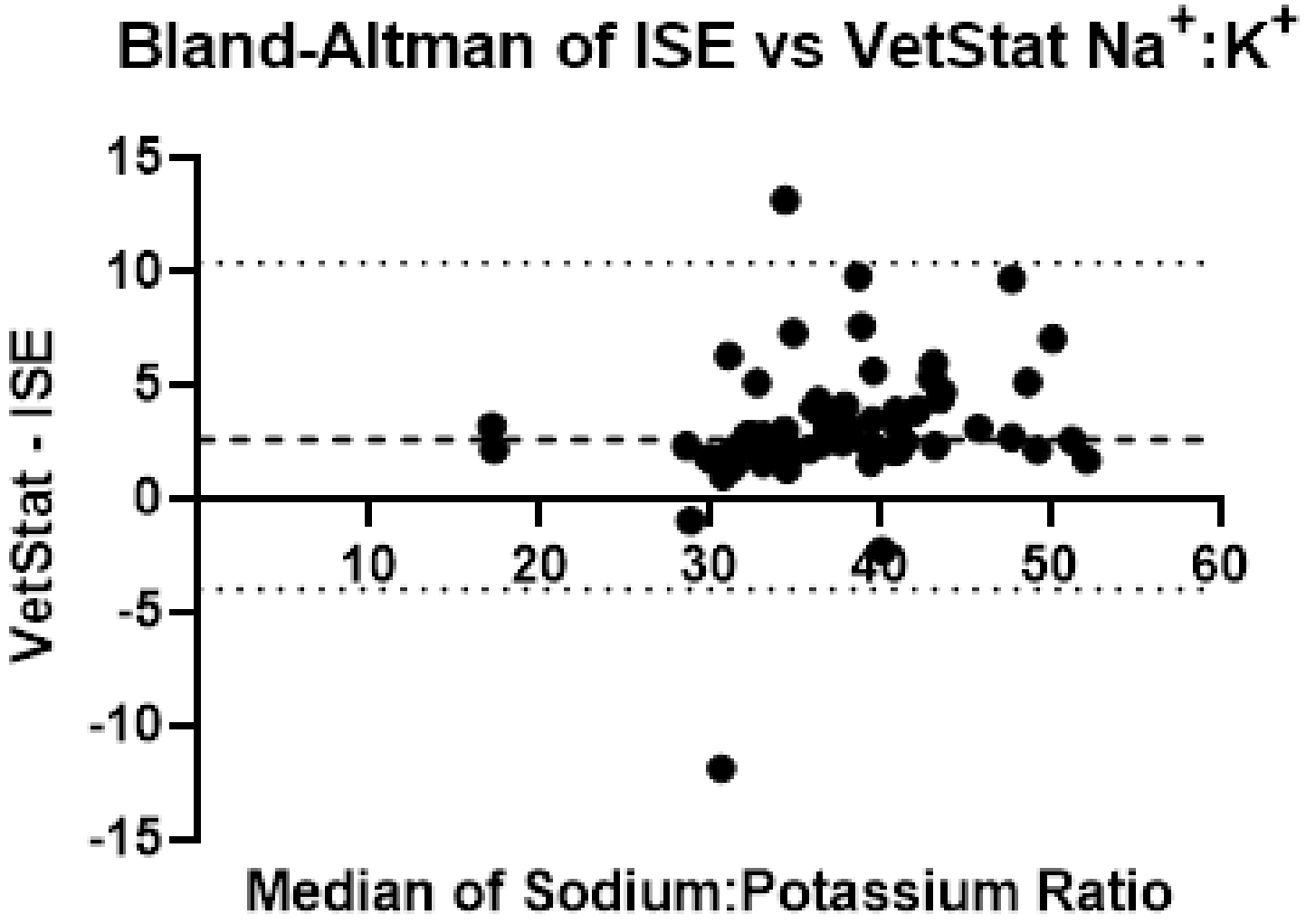

Bland-Altman of ISE vs VetStat Na+:K+
VetStat - ISE
15
10
5
0
-5
-10
-15
10
20
30
40
50
60
Median of Sodium:Potassium Ratio

Figure 5 – Cohen's Kappa coefficient analysis of the IDEXX VetStat® compared to the reference ISE method.

Frequencies

N	72

	Vetstat Na L/N/H			
ISE Na L/N/H	Low	Normal	High	Total
Low	3	1	0	4
	4.2%	1.4%	0.0%	5.6%
Normal	1	49	17	67
	1.4%	68.1%	23.6%	93.1%
High	0	0	1	1
	0.0%	0.0%	1.4%	1.4%
Total	4	50	18	72
	5.6%	69.4%	25.0%	

Agreement

Kappa	0.24
Wald 95% CI	0.03 to 0.45
SE	0.105

Frequencies

N	72

	Vetstat K L/N/H			
ISE K L/N/H	Low	Normal	High	Total
Low	6	0	0	6
	8.3%	0.0%	0.0%	8.3%
Normal	3	61	0	64
	4.2%	84.7%	0.0%	88.9%
High	0	0	2	2
	0.0%	0.0%	2.8%	2.8%
Total	9	61	2	72
	12.5%	84.7%	2.8%	

Agreement

Kappa	0.82
Wald 95% CI	0.63 to 1.00
SE	0.099

Frequencies

N	72

	Vetstat Cl L/N/H			
ISE Cl L/N/H	Low	Normal	High	Total
Low	1	0	0	1
	1.4%	0.0%	0.0%	1.4%
Normal	3	62	1	66
	4.2%	86.1%	1.4%	91.7%
High	0	5	0	5
	0.0%	6.9%	0.0%	6.9%
Total	4	67	1	72
	5.6%	93.1%	1.4%	

Agreement

Kappa	0.14
Wald 95% CI	-0.17 to 0.45
SE	0.160

Frequencies

N	72

	VetStat Na:K L/N/H			
ISE Na:K L/N/H	Low	Normal	High	Total
Low	2	0	0	2
	2.8%	0.0%	0.0%	2.8%
Normal	1	40	12	53
	1.4%	55.6%	16.7%	73.6%
High	0	1	16	17
	0.0%	1.4%	22.2%	23.6%
Total	3	41	28	72
	4.2%	56.9%	38.9%	

Agreement

Kappa	0.60
Wald 95% CI	0.42 to 0.78
SE	0.091

Frequencies

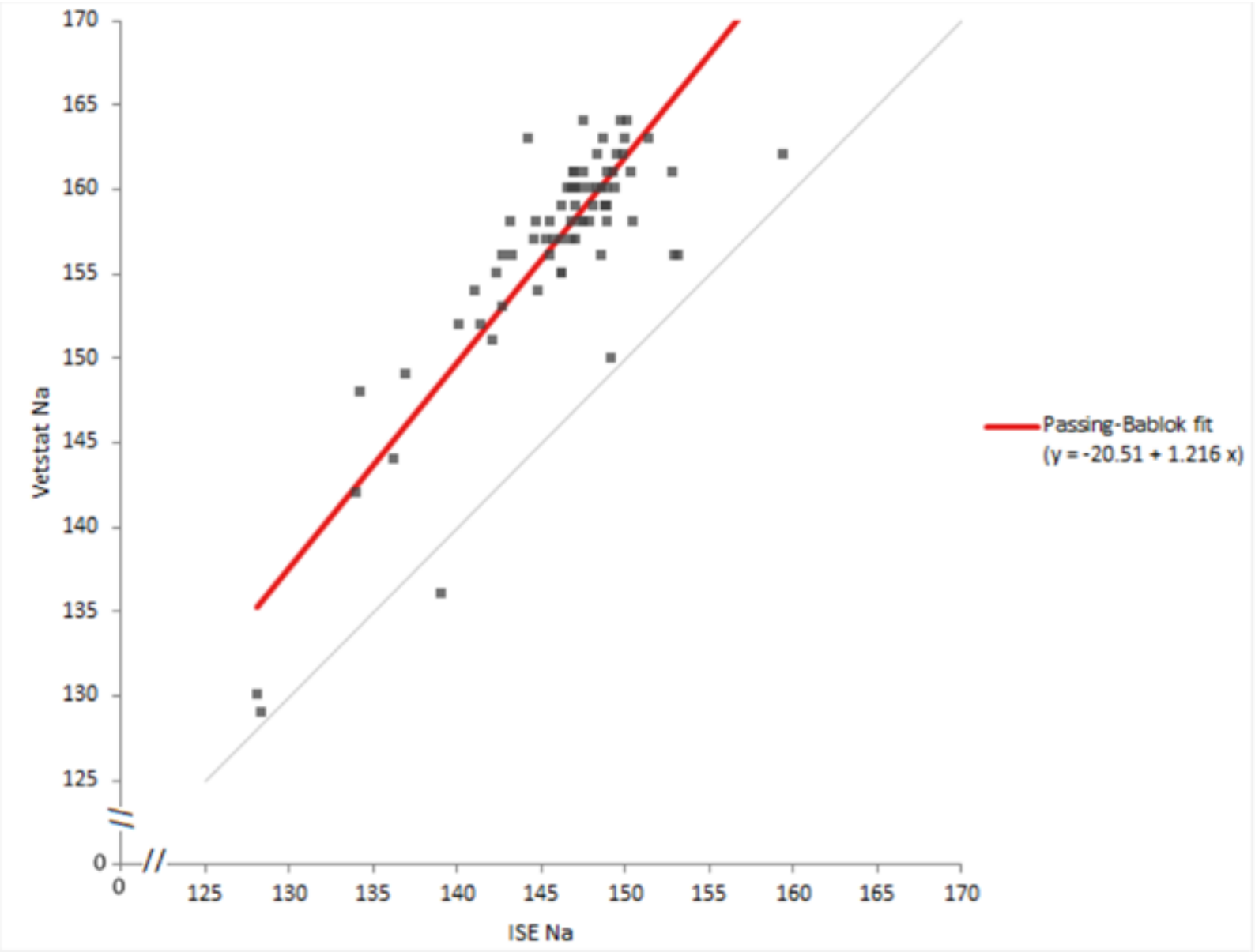
N	72

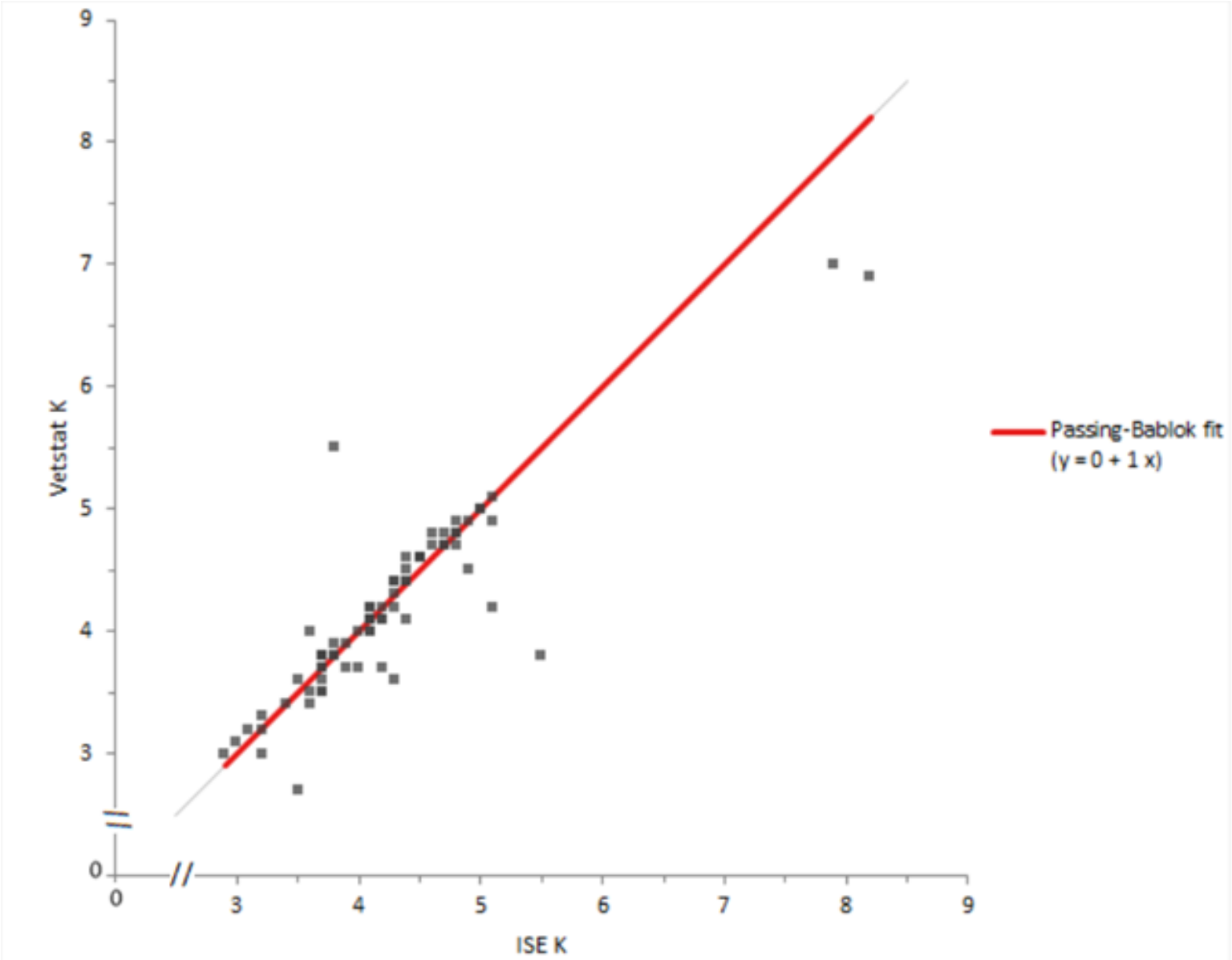
	VetStat Na:K 27-32 L/N/H			
ISE Na:K 27-32 L/N/H	Low	Normal	High	Total
Low	2	0	0	2
	2.8%	0.0%	0.0%	2.8%
Normal	0	7	13	20
	0.0%	9.7%	18.1%	27.8%
High	1	0	49	50
	1.4%	0.0%	68.1%	69.4%
Total	3	7	62	72
	4.2%	9.7%	86.1%	

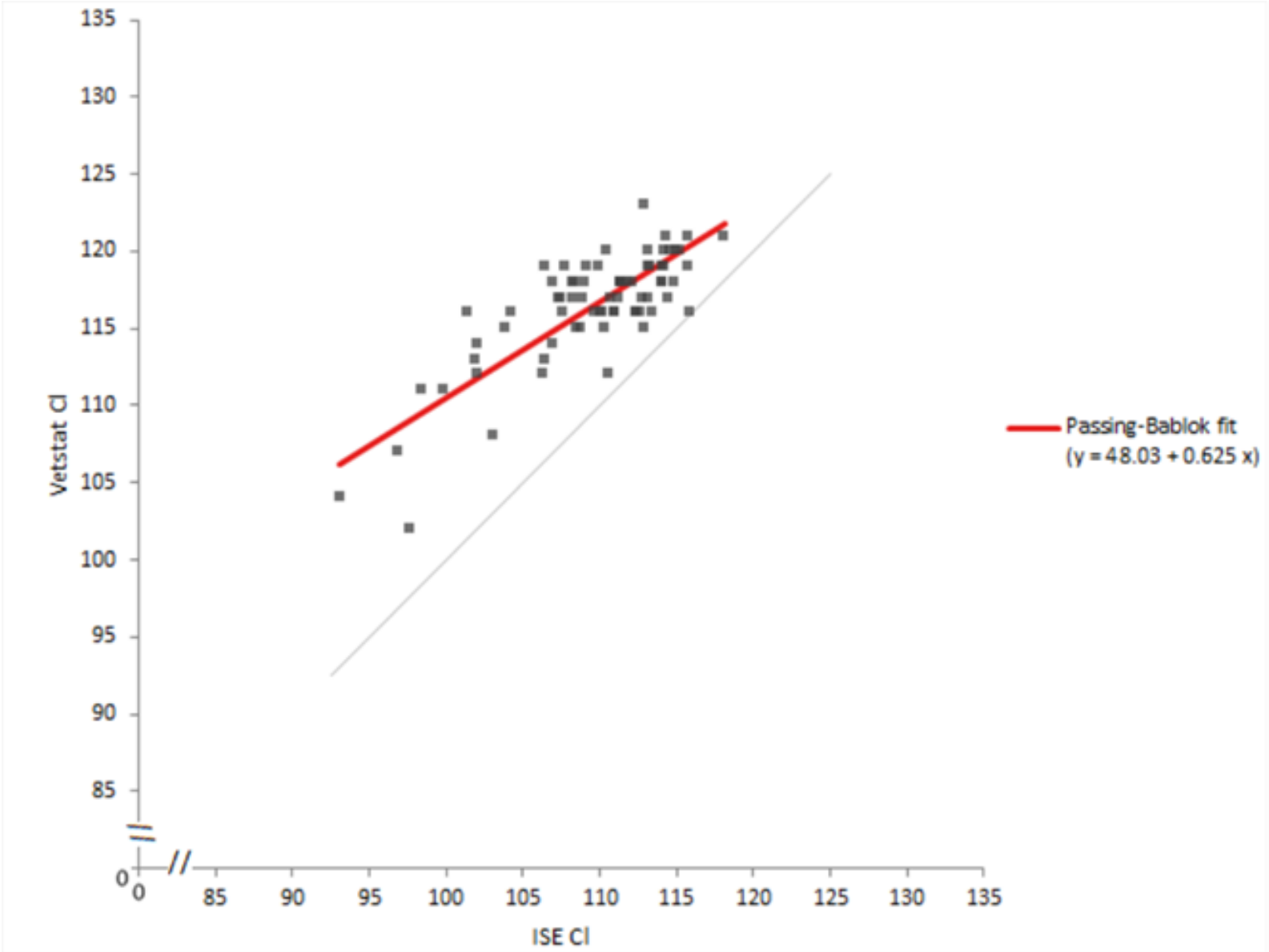
Agreement

Kappa	0.48
Wald 95% CI	0.26 to 0.70
SE	0.111

Figure 6 – Passing-Bablok regression analysis of the IDEXX VetStat® compared to the reference ISE method.




Vetstat K
ISE K
Passing-Bablok fit
(y = 0 + 1 x)
0
3
4
5
6
7
8
9


135
130
125
120
115
110
105
100
95
90
85
0
0
85
90
95
100
105
110
115
120
125
130
135
Vetstat Cl
ISE Cl
Passing-Bablok fit
(y = 48.03 + 0.625 x)

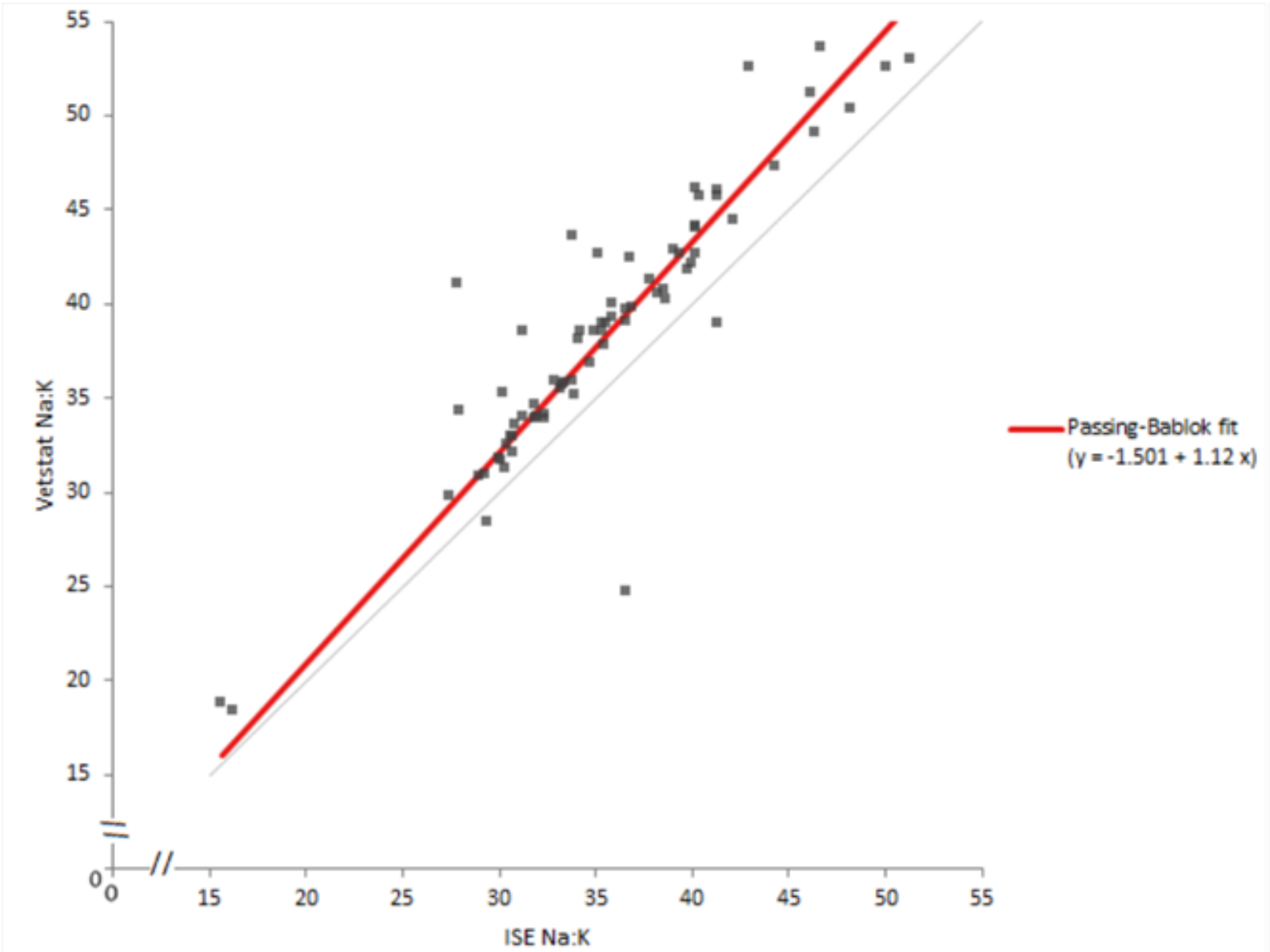

55
50
45
40
35
30
25
20
15
0
0
15
20
25
30
35
40
45
50
55
Vetstat Na:K
ISE Na:K
Passing-Bablok fit
(y = -1.501 + 1.12 x)

Table 6 – Summary of Passing-Bablok regression analysis of the IDEXX Catalyst™ Dx and IDEXX VetStat ® analysers compared to the reference ISE method.

Catalyst						
	y intercept			Slope		
Analyte	Estimate	95% CI	Constant Bias	Estimate	95% CI	Proportional Bias
Na^+	-35.74	-57.86 to -17.32	Yes	1.28	1.15 to 1.43	Yes
K^+	0.07	-0.325 to 0.2	No	1.03	1 to 1.13	No
Na^+: K^+	-0.37	-2.0 to 1.68	No	1	0.94 to 1.05	No
Cl^-	14.36	6.03 to 22.38	Yes	0.89	0.82 to 0.97	Yes
VetStat						
	y intercept			Slope		
Analyte	Estimate	95% CI	Constant Bias	Estimate	95% CI	Proportional Bias
Na^+	-20.51	-55.64 to 11.64	Yes	1.22	1 to 1.46	Yes
K^+	0	-0.24 to 1.09	No	1	1 to 1.06	No
Na^+: K^+	-1.5	-5.13 to 1.3	No	1.12	1.04 to 1.23	Yes
Cl^-	48.03	32 to 63	Yes	0.63	0.49 to 0.77	Yes

Key: CI = Confidence Interval.

Constant bias was present if the 95% CI for the Y intercept of the regression line did not include 0.

Proportional bias was present if the 95% CI for the slope of the regression line did not include 1

3.6 Total Observed Error Analysis

Both the IDEXX Catalyst and VetStat analysers did not meet the precision targets for sodium, potassium and chloride with $TE_{(Obs)}$ higher than the TE_A in all cases other than the chloride measured on the VetStat which was just acceptable (Table 7). The coefficient of variation (CV) for all analytes was relatively small on both analysers so this failure was mainly due to the large bias that was present in both machines.

The clinical relevance of this disagreement between methods was investigated by assessing how often the POC analysers produced discordant results (i.e. results which fell outside their normal reference interval on a POC analyser when the ISE method found them to be "normal" and within the reference interval or vice versa). For the Na^+: K^+ ratio, the narrower range of 27 to 32 was used as this is recommended for the adjustment of Zycortal® (DOCP) doses.

With the Catalyst analyser, discordant results were produced in 21 cases (6%) for sodium concentrations, 27 cases (8%) for potassium concentrations, 46 cases (14%) for chloride concentrations and 47 cases (14%) for the Na^+: K^+ ratio.

The VetStat analyser meanwhile produced results which disagreed with the ISE method in 19 cases (26%) for sodium concentrations, 3 cases (4%) for potassium concentrations, 9 cases (13%) for chloride concentrations and 14 cases (19%) for the Na^+: K^+ ratio.

Table 7 – Summary of the intra-assay coefficient of variation, bias and observed total error of the IDEXX Catalyst ™Dx and IDEXX VetStat ® analysers.

		Catalyst			VetStat		
Analyte	TE_A	CV%	Bias %	$TE_{(Obs)}$ %	CV%	Bias %	$TE_{(Obs)}$ %
Na^+	5%	1.3 (1.1 to 1.6)	3.5 (-2.3 to 5.3)	6.1 (4.7 to 7.6)	0.6 (0.3 to 0.8)	7.6 (-10.6 to -4.2)	8.7 (4.8 to 11.7)
K^+	5%	2.5 (2.3 to 2.7)	9.0 (-17.0 to -3.3)	14.0 (8.4 to 21.6)	1.2 (1.0 to 1.6)	4.7 (-7.7 to 1.7)	7.2 (3.7 to 11.0)
Cl^-	5%	1.8 (1.0 to 2.9)	3.5 (-5.2 to 0.8)	7.2 (2.9 to 10.9)	0.5 (0.4 to 0.5)	4.2 (-7.2 to -1.3)	5.0 (2.2 to 8.0)

Key: CV= Coefficient of Variation

$TE_{(Obs)}$= Observed Total Error

3.7 Sensitivity and Specificity

The sensitivity and specificity of both POC analysers was investigated relative to their ability to detect electrolyte concentrations that fell outside their respective reference intervals compared to that of the reference laboratory indirect ISE method (Table 8). The Catalyst analyser was relatively poorly sensitive detecting hypernatremia, hyperchloremia and hypokalaemia but had good specificity. The poor sensitivity was partly because no samples had increased sodium concentrations and very few had increased chloride levels however the poor sensitivity for hypokalaemia is more concerning clinically.

The VetStat analyser had poor sensitivity for detecting low sodium and high chloride concentrations. Again, this was likely due to low sample numbers with these changes reducing the confidence intervals. The specificity for detecting high sodium concentrations was poorer than those of other electrolyte abnormalities on this analyser.

Table 8 – Summary of the sensitivity and specificity of the IDEXX Catalyst ™ Dx and IDEXX VetStat® analysers with 95% Confidence Intervals.

	Catalyst			Vetstat		
ISE Analyte	Sensitivity	Specificity	AUC	Sensitivity	Specificity	AUC
Hypernatremia (>159 mmol/L)	0.00 (0.00 to 0.79)	0.96 (0.94 to 0.98)	0.52	1.00 (0.34 to 1.00)	0.76 (0.65 to 0.85)	0.88 (0.83 to 0.93)
Hyponatremia (<136 mmol/L)	0.92 (0.67 to 0.99)	0.98 (0.96 to 0.99)	0.95 (0.88 to 1.03)	0.75 (0.30 to 0.95)	0.99 (0.92 to 1.00)	0.87 (0.62 to 1.11)
Hyperkalaemia (>5.8 mmol/L)	1.00 (0.61 to 1.00)	0.98 (0.96 to 0.99)	0.99 (0.98 to 1.0)	1.00 (0.34 to 1.00)	1.00 (0.95 to 1.00)	1.0
Hypokalaemia (<3.4 mmol/L)	0.33 (0.17 to 0.55)	0.98 (0.96 to 0.99)	0.66 (0.55 to 0.76)	1.00 (0.61 to 1.00)	0.96 (0.88 to 0.98)	0.98 (0.95 to 1.0)
Hyperchloremia (>115 mmol/L)	0.23 (0.12 to 0.39)	1.00 (0.99 to 1.00)	0.61 (0.54 to 0.69)	0.00 (0.00 to 0.39)	0.99 (0.92 to 1.00)	0.51 (0.49 to 0.52)
Hypochloraemia (<95 mmol/L)	1.00 (0.61 to 1.00)	0.94 (0.91 to 0.96)	0.97 (0.96 to 0.98)	1.00 (0.21 to 1.00)	0.96 (0.88 to 0.99)	0.98

Key: AUC = Area Under Curve

4 Discussion

4.1 Study Design

This study achieved the ASVCP recommendation for method comparison studies as more than 40 paired samples were tested on both POC analysers. However, not all these samples were truly independent as multiple dogs were tested numerous times. This was not a problem for the IDEXX Catalyst as 45 different dogs were included however the IDEXX VetStat only achieved 29 individual dogs.

The precision analysis also reached the ASVCP recommendation of five replications for intra-assay (within run) variation however this could have been more robust by also being performed over five consecutive days to ascertain any inter-assay (between run) changes.

4.2 Study Results

4.2.1 Study Population

The study population was very diverse with a range of different ages, breeds and therefore weights of dogs and equal numbers of each sex. Some dogs presented were newly diagnosed with hypoadrenocorticism and had just been transitioned onto long term treatment whilst others had been stable on treatment for many months or years. This makes it representative of dogs with hypoadrenocorticism seen in clinical practice. There was no effect of individual dogs, age, breed, sex, nor what medications it was receiving.

4.2.2 Point-of-care Analyser Performance

The concentrations of sodium, potassium and chloride measured by the Catalyst and VetStat analysers, as well as the calculated Na^+: K^+ ratios, are not interchangeable with those from a reference laboratory analyser using an indirect ISE method. Cohen's Kappa coefficient analysis was performed to assess the agreement between the POC analysers using analyser specific reference intervals and found that chloride κ values were only fair or poor whilst sodium analysis performed only slightly better, being classified as moderate or fair. Potassium analysis on the other hand was more reliable and classified as very good on the VetStat analyser. Some variation was expected given the different methodology involved with each analyser; however, the magnitude especially of the difference in sodium concentrations measured, is clinically significant in dogs treated for hypoadrenocorticism. Electrolyte derangements are common with many disease processes and it is very important that POC analysers accurately measure electrolytes across a range of concentrations.

Hypoadrenocorticism is often diagnosed in dogs with low Na^+: K^+ ratios however low ratios are also frequently found in many other disease conditions, especially those affecting the urogenital, cardiorespiratory and gastrointestinal systems (Nielsen et al., 2008). The data sheet for Zycotal® (25mg/ml, Dechra Ltd.), the formulation of DOCP licensed to treat dogs with hypoadrenocorticism in the European Union (European Medicines Agency, 2019), relies on the use of Na^+: K^+ ratios for dose adjustments. The Na^+: K^+ ratios produced on these POC analysers were often different when compared to those from the reference laboratory indirect ISE method but not consistently (i.e. sometimes they were higher and sometimes lower). This study found that there were many instances in which treatment decisions would have been different depending on which analyser was used. This was also true when the sodium and potassium concentrations themselves were examined in relation to the analysers reference intervals. This study found that in up to 14% of Catalyst samples and 26% of VetStat samples, different and potentially deleterious treatment decisions could be made which is clinically relevant. In this study that would have meant under or overdosing dogs with DOCP, but these results are likely also true of dogs with conditions other than hypoadrenocorticism. Previous studies have shown other POC analysers, utilising a different methodology (direct ISE),also have poor agreement and correlation when measuring electrolytes (West et al., 2014, Uyanik et al., 2015).

In a survey of veterinary practices, POC analysers manufactured by IDEXX accounted for 85% of all in practice analysers and more than two thirds (71%) of respondents reported that they used the reference intervals supplied by the manufacturer without further adjustment or assessment (Bell et al., 2014). The ASVCP recommend that reference intervals supplied by instrument manufacturers are validated to ensure that they are suitable for the population which they will be testing (Flatland et al., 2013). It is recognised that using an instrument of the same or similar analytical methodology may provide different results due to differences in patient population and operator and site-specific differences. It is recommended that validation be pursued using at least 20 patient samples as detailed in the ASVCP reference interval guidelines (Friedrichs et al., 2012). This lack of instrument specific reference intervals for the Catalyst and VetStat analysers likely accounts for their poor performance in this study.

The results of this study can, strictly, only be directly applied to the individual IDEXX Catalyst™ Dx and VetStat® analysers which were tested in the Small Animal Hospital, University of Glasgow and do not necessarily apply to all analysers from this manufacturer, those of the same model or of other analysers utilising these methods from different manufacturers. However, it is likely that the general findings are applicable and highlight that caution should be applied when interpreting electrolyte concentrations from POC analysers. A previous study has documented variations in the diagnostic performance between analysers of the same model as one of those used in this study (IDEXX Catalyst™ Dx) as well as many others not tested (Rishniw et al., 2012). Therefore, each individual analyser's performance should be evaluated and ideally the same analyser be used for all repeated analysis. This may be difficult however when model specific reference intervals are produced by the manufacturer as with the IDEXX Catalyst™ Dx (IDEXX Laboratories, 2015) and IDEXX VetStat® (IDEXX Laboratories, 2010).

4.2.3 Precision Analysis

Both POC analysers provided unacceptable electrolyte concentration results on repeated analysis according to the ASCVP TE_A limits. No investigation of inter-assay variation was performed so it is possible that significant CV and bias also existed between runs. Both POC analysers had regular quality control tests performed and it is likely that this was more often than would be performed in a first opinion practice given the study's setting in a busy teaching hospital.

4.3 Limitations

4.3.1 Case Selection

Only dogs with hypoadrenocorticism were included in this study. Whilst it is felt unlikely, there could be an unidentified substance (i.e. matrix effect) in dogs with hypoadrenocorticism which is not present in healthy dogs or dogs with other disease conditions which could have interfered with electrolyte analysis. Some caution should therefore be applied when extrapolating the results from this study to other canine diseases and especially to conditions in other species. However, this is representative of clinical practice and is hopefully mitigated to a degree by excluding dog's with multiple diseases. Additionally, some dogs were included multiple times whilst others were included only once on each analyser. Some dogs may have had some unknown quality which affected analysis by one method and not another; however, this was felt to be unlikely and would not be accounted for in clinical practice. Linear regression did not identify any effect of individual dog, age, sex, treatment (DOCP and prednisolone vs fludrocortisone) and sample number.

4.3.2 Cortisol Assays

The European Society of Veterinary Endocrinology runs the voluntary European Veterinary Endocrine Quality Assurance Scheme (EVE-QAS). This aims to promote high quality and accurate hormonal laboratory testing which is consistent between laboratories. At the last test release, cortisol testing had a CV of 13% between the laboratories involved (European Society of Veterinary Endocrinology, 2019). Laboratories which have signed up to such a scheme are more likely to engage in regular quality assurance and use validated methods. Most of the dogs diagnosed with hypoadrenocorticism in this study were diagnosed in first opinion practise. Therefore, different external laboratories were used potentially employing different cortisol assays run on different analysers with different reference intervals compared to the Veterinary Diagnostic Service used by the Small Animal Hospital which is a member of the EVE-QAS.

4.3.3 Sample Ranges

Given the population of dogs which were studied, there were no samples with a sodium concentration which fell above the upper reference interval of the reference indirect ISE method. It is therefore possible that different biases may exist in this untested zone which were not identified in this study. This also limited the sensitivity and specificity analysis of these analysers. Hypernatremia is less frequently encountered in clinical veterinary practice than hyponatremia with a 5.7% incidence rate versus 25.5% for hyponatremia (Ueda et al., 2015). Future studies would need to examine confirmed cases of hypernatremia compared to normal controls.

4.3.4 Sample Numbers

Fewer samples were run on the IDEXX VetStat® compared to the IDEXX Catalyst™ Dx. Ideally the same number of samples would have been run on both machines with samples run in triplicate i.e. on both IDEXX analysers and at the reference laboratory. This was not possible as it would have required collecting more blood than was clinically necessary and funding was not available for this additional testing.

4.3.5 Treatments

The dogs included in this study were either treated with DOCP and prednisolone or with fludrocortisone. Some dogs were treated with both treatments at different time points. Ideally only one treatment regime would have been used throughout to remove this confounding factor however linear regression did not identify an effect of treatment.

4.3.6 Study Duration

This study took place over a long period of time and some change in analyser performance may have been expected. This is a recognised problem that potentiometry based methods can suffer from degradation of the ISE itself, eventually requiring replacement. In contrast, the methods used in POC analysers, generally do not require routine periodic replacement of equipment. However, this more closely represents clinical practice as most veterinary practices keep their POC electrolyte analyser for many years and often service them themselves rather than by the manufacturer (Bell et al., 2014). There was no evidence of change over time from our data which may have been due to the regular servicing and quality control procedures within our hospital.

4.3.7 Change in Laboratory Analyser

The change in analyser from the Olympus AU640 to the Siemens Dimension Xpand Plus was unfortunate but unavoidable during this study. In a clinical setting, a reference laboratory changing their analyser would go unnoticed by the submitting veterinary surgeon and it was ensured that the two analysers were producing comparable results before the switch was made. Additionally, analysis of the samples from the first and last three dogs tested on the reference analyser (i.e. the Olympus AU640 at the start compared to the Siemens Dimension Xpand Plus at the end), showed no significant change during the study period. This study may have been more robust if this change had not been made but the change may have increased the relevance of the study being more comparable to clinical practice.

4.3.8 Other Clinical Pathology Parameters

Routine biochemistry and haematology testing were not performed at the same time as each electrolyte sample during this study and it is possible that some of the dogs may have had altered PCV, protein or lipid levels during some visits. These physiological abnormalities may have impacted electrolyte analysis and varied between samples. This represents normal clinical practice and should have been accounted for to a degree by using paired samples. However, data from the 30 of the dogs included in the clinical trial that ran alongside this study does not suggest this was a problem. This trial compared dogs treated with DOCP and prednisolone to those treated with fludrocortisone alone. On haematology, it found an increase in neutrophil count in dogs treated with DOCP and prednisolone, likely due to exogenous glucocorticoids causing a “stress leukogram”. On biochemistry, the urea and creatinine levels were lower in dogs treated with DOCP and prednisolone(Spence, 2020).

4.3.9 Samples Tested

The IDEXX VetStat® analyser tests were performed on heparinised whole blood rather than plasma as with the IDEXX Catalyst™ Dx and laboratory indirect ISE method. It is possible that this caused a difference in the analysis and future studies should look at any difference between electrolytes analysed in whole blood, plasma and serum on this analyser. It is well documented that whole blood and plasma samples invariably contain slightly lower potassium levels (0.1 to 0.7 mmol/L) compared to serum depending on platelet count, however the effect of whole blood versus plasma is less clear. Most reference laboratory intervals for serum potassium are 0.2 to 0.5 mmol/L higher than those for plasma (Schindler et al., 2018).

4.4 Future Studies

Further work is needed to assess the IDEXX Catalyst™ Dx and IDEXX VetStat® analysers between day variation. Future studies should also aim to assess electrolyte analysis in a range of other medical conditions, over a wider range of sodium, potassium, chloride and Na^+: K^+ ratios. Additionally, different POC analysers should be investigated especially those using different methodologies (e.g. direct ISE).

4.5 Conclusion

This study demonstrates that there are inherent differences between the electrolyte concentrations measured by the IDEXX Catalyst™ Dx and IDEXX VetStat® analysers and a reference laboratory using an indirect ISE method in dogs with hypoadrenocorticism. Therefore, the null hypotheses were rejected.

4.6 Clinical Implications

As a result, it is important that clinicians do not to rely on the electrolyte values produced by these analysers at the expense of other clinical findings. It is suggested that the same analyser be used for all dose adjustments and repeated electrolyte analysis, with attention paid to the individual reference interval for that machine. Additionally, clinicians should take steps to ascertain how their electrolyte analyser compares to their reference analyser in cases where comparisons are made.

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