PAPER

Measurement of thyroxine and cortisol in canine and feline blood samples using two immunoassay analysers

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Objectives: The AIA-360 (Tosoh Corporation) is an automated immunoassay analyser. The aims of this study were to estimate the precision of thyroxine and cortisol AIA-360 immunoassays in canine and feline samples and to compare the results produced with those obtained by a chemiluminescence analyser (Immulite® 1000, Siemens).

Methods: Blood samples from 240 clinical cases (60 dogs and 60 cats for both thyroxine and cortisol) were analysed using both instruments.

Results: Deming regression calculations showed excellent correlation (thyroxine, canine r_s=0·94, feline r_s=0·97; cortisol, canine r_s=0·97, feline r_s=0·97). Agreement between the two instruments was examined by Bland–Altman difference plots, which identified wide confidence intervals and outliers for thyroxine (canine n=6, feline n=4) and cortisol (canine n=3, feline n=4) results. Inter/intra-run precision of the AIA-360 was excellent for both cortisol and thyroxine (coefficients of variation <7%).

Clinical Significance: The instrument showed excellent correlation for cortisol and thyroxine in canine and feline samples demonstrating that the AIA-360 can be used in clinical practice. The agreement studies suggest that the results from the AIA-360 cannot be used interchangeably with those generated by the Immulite 1000 and should be interpreted using reference intervals that have been established specific to the AIA-360.

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INTRODUCTION

There have been many technological advances in veterinary practice in the last two decades and most clinics now have the ability to perform laboratory analyses in-house. The most widely used in-house instruments are haematology (Dewhurst *et al.* 2003, Becker *et al.* 2008, McDaniel *et al.* 2013) and biochemistry analysers (Sutton *et al.* 1999, Trumel *et al.* 2005, Papasouliotis *et al.* 2006, 2008), which generate results immediately, aiding in the diagnosis of various diseases.

Feline hyperthyroidism and canine hypothyroidism, hyperadrenocorticism and hypoadrenocorticism are commonly diagnosed in veterinary practice. Although these endocrinopathies are associated with various haematological and biochemical abnormalities, measurement of total thyroxine (T4) and cortisol is necessary for the establishment of a final diagnosis. The most common methodology used for the measurement of T4 and cortisol is a non-radioactive chemiluminescent immunoassay (Kemppainen & Birchfield 2006, Lennon *et al.* 2007, Russell *et al.* 2007). However, the analysers that employ chemiluminescence are expensive to purchase and maintain, require a high test volume for economic viability and are technically demanding. For such reasons, these instruments are used by commercial veterinary laboratories and are not suitable for in-house use.

Measurement of canine and feline T4 and cortisol can be performed in-house using a commercially available ELISA kit (Snap®, Idexx Laboratories) and analysing equipment (Snap® Reader, Idexx Laboratories) (Lurye *et al.* 2002, Kintzer & Turgeon 2005, Kemppainen & Birchfield 2006), but information on the use of automated analysers for measuring these hormones in veterinary clinics is not available and there are no published studies at the time of writing.

Recently, an automated immunoassay analyser, originally designed for measurement of various analytes in humans (AIA-360®, Tosoh Bioscience), has become available for use in the veterinary market. The aims of this study were (1) to estimate the precision of the T4 and cortisol AIA-360 immunoassays and (2) to compare the results produced by the AIA-360 with those obtained by a chemiluminescence analyser (Immulite® 1000, Siemens) that is used routinely in the authors' laboratory.

MATERIALS AND METHODS

Clinical cases

Blood samples from 120 clinical cases (60 dogs and 60 cats), submitted to the Diagnostic Laboratories, Langford Veterinary Services during January and February 2013, were included in this study. Samples were utilised from clinical cases to generate a wide range of values. This can provide more accurate information regarding the degree of correlation and agreement between the two instruments (Jensen & Kjelgaard-Hansen 2006). The animals showed a variety of clinical signs and had all been referred to the Small Animal Referral Hospital, Langford Veterinary Services for further investigation of their illnesses. During these investigations, venous blood samples (2 to 3 mL) were collected into plain tubes. Following clot retraction and centrifugation, harvesting of serum and performance of requested biochemical analyses, any excess serum (≥750 µL) was placed into plain tubes and stored at –20°C for 1 to 7 days. On the day of testing, frozen samples were thawed using a warm bath; T4 (nmol/L) and cortisol (nmol/L) were then measured using both analysers. Samples with pronounced haemolysis or lipaemia were excluded. Six samples in total appeared mildly haemolysed and five samples mildly lipaemic. The lipaemic samples were successfully cleared following "hard" centrifugation (1751 g for 10 minutes).

The samples were run once on each instrument, utilising reagents from the same batch for each analyser and according to the manufacturer's instructions (Immulite® 1000 Operator's Manual and TOSOH AIA-360® System Operator's Manual).

Clinically healthy animals

T4 and cortisol were also measured in serum samples from clinically healthy dogs (n=66) and cats (n=27) using the AIA-360 analyser as described above. These animals were registered blood donors or were undergoing elective surgery. The samples used were surplus serum taken as part of an established routine health check. This excess serum had been frozen at –20°C for 3 to 5 months. All surplus stored samples were used with owners' consent.

Immulite• 1000

This analyser utilises solid-phase, competitive chemiluminescence immunoassays that have been marketed for use in humans and validated for use in dogs and cats (Singh *et al.* 1997).

Routine maintenance, instrument preparation, setup, adjustment, assay and quality control procedures were performed as defined in the Operator's Manual. Serum samples (150 µL) were placed in the test cup and the first results were available after 30 minutes. The validated human assays were employed for measuring cortisol in both species and T4 in cats. In dogs, T4 was measured by the Immulite® canine total T4 assay.

The lower (upper) reported limits for the human and canine T4 assays were 12·9 (309·0) and 6·4 (193·0) nmol/L, respectively. For the cortisol assay the lower limit was 28·0 and the upper limit was 1380·0 nmol/L. The reference intervals established at this laboratory for canine T4 and basal cortisol were 10·0 to 40·0 and 30·0 to 215·0 nmol/L, respectively. For feline T4 the reference interval was 15·0 to 60·0 nmol/L and for basal cortisol less than 28·0 to 200·0 nmol/L.

The accuracy of the methodologies was assessed by continuous bimonthly participation in an external quality assurance programme (RIQAS Immunoassays; Randox).

AIA-360•

This analyser utilises a competitive fluorescent enzyme immunoassay, which is performed entirely within small, single-use test cups containing all necessary reagents. The analyte present in the sample competes with enzyme-labelled hormone for a limited number of binding sites on hormone-specific antibodies immobilised on magnetic beads. The beads are washed to remove the unbound enzyme-labelled hormone and then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labelled hormone that binds to the beads is inversely proportional to the hormone concentration in the test sample.

Calibration, daily check and maintenance procedures were carried out as described in the System Operator's Manual. Accuracy performance data for human T4 and cortisol, including analyte recovery and dilutional studies, had been previously evaluated and were available in the manufacturer's technical bulletins (ST AIA-Pack Cort, page 2; ST AIA-Pack T4, page 2). The time to generate the first result was 20 minutes with one new result every minute thereafter.

Each serum sample (150 µL) was placed in the test cup and both hormones were measured by the human immunoassays. The lower (upper) reported values for the T4 and cortisol assays were 6·4 (309·0) and 28·0 (1656·0) nmol/L, respectively.

The manufacturer provided reference intervals for canine (13·0 to 52·0 nmol/L) and feline T4 (10·0 to 55·0 nmol/L) but only for feline basal cortisol (30·0 to 270·0 nmol/L).

Within-run precision studies were performed by measuring the hormones in the same sample 10 times in the same analysis. Pooled canine and feline samples were used creating three samples with one each of relatively low, medium or high concentrations of T4 or cortisol.

Between-run precision was estimated by analysing the same sample once on 10 consecutive working days; again three pooled

canine and feline samples were used, which contained relatively low, medium or high concentrations of T4 or cortisol.

Statistical analysis

When a result was lower than the measurable limit and reported as "less than," the value that was used for statistical analysis was calculated by the midpoint between zero and the lower limit of measurement [(0+lower limit reported value)/2]. For canine T4 this was 3·2 (<6·4), feline T4 was 6·45 (<13·7) and canine and feline cortisol was 14·0 (<28·0) nmol/L.

Bland–Altman difference plots were performed using the Prism® 4 (Graphpad software Inc.) statistical programme for Macintosh. Deming's regression analysis and calculation of coefficient of variation (CV) were performed using the SPSS® 19.0 Statistics (IBM Inc.) programme for Windows.

Deming regression and Spearman's correlation coefficient (r_s) were used to determine the correlation between the two analysers for each of the hormones in both species. Previous similar studies have utilised an objective system to characterise correlations based on the r_s values as excellent ($r_s \geq 93$), good (r_s=0·80 to 0·92), fair (r_s=0·59 to 0·79) or poor ($r_s \leq 0·59$) (Dewhurst *et al.* 2003, Papasouliotis *et al.* 2006, 2008).

Bland–Altman difference plot analysis was performed to determine the degree of agreement between the two analysers. Agreement was considered good when there was no real bias or the bias (mean of the differences, AIA-360®–Immulite® 1000) was small, the 95% confidence intervals (CIs) for the bias were narrow and no outliers were present [no values fell outside the limits of agreement (mean of the differences ±2 sd)]. No real bias was indicated when the 95% CI for the bias included zero (Bland & Altman 1986, Jensen & Bantz 1993, Gray *et al.* 1999).

Within- and between-run precisions were expressed as CV (%) following calculation of the mean and standard deviation (sd) for each set of results.

RESULTS

T4

Precision estimates for T4 using the AIA-360 analyser were conducted on pooled canine and feline serum samples. For the canine studies, the low, medium and high samples contained 15·0 to 30·0 (mean: 23·7), 40·0 to 55·0 (mean: 49·6) and 60·0 to 80·0 (mean: 79·3) nmol/L. For the feline studies the samples contained 15·0 to 25·0 (mean: 21·4), 40·0 to 65·0 (mean: 57·9) and 70·0 to 120·0 (mean: 105·0) nmol/L. Results revealed that the mean within- and between-run CVs for T4 were ≤7% (Table 1).

Correlations between the AIA-360 and Immulite results were excellent (Table 2, Fig 1A, B). Examination of the Bland–Altman difference plots revealed that there was no real bias for canine or feline results; however, the 95% CIs for the bias were wide and 10 results (6 canine and 4 feline) were identified as outliers (Table 3, Fig 2A, C).

Cortisol

Precision estimates for cortisol using the AIA-360 analyser were conducted on pooled canine and feline serum samples. For the canine studies, the low, medium and high samples contained 70·0 to 130·0 (mean: 105·0), 200·0 to 350·0 (mean: 275·6) and 390·0 to 530·0 (mean: 444·8) nmol/L, respectively. For the feline studies the samples contained 40·0 to 50·0 (mean: 43·0), 120·0 to 200·0 (mean: 170·1) and 220·0 to 350·0 (mean: 278·0) nmol/L, respectively. Results revealed that the average within- and between-run CVs for cortisol were less than 5% (Table 1).

Correlations between the AIA-360 and Immulite were excellent (Table 2, Fig 1C, D). Bland–Altman difference plots demonstrated that there was no real bias but the 95% CIs for the bias were wide and seven results (three canine and four feline) were characterised as outliers (Table 3, Fig 2B, D).

Reference intervals for the AIA-360

In clinically healthy dogs (n=66), the T4 results ranged from 10·3 to 43·3 nmol/L and the cortisol results from less than 28·0 to 251·0 nmol/L. The calculated reference interval (mean ±2 sd) for T4 was 12·0 to 45·0 nmol/L and for cortisol was less than 28·0 to 186·0 nmol/L.

In clinically healthy cats (n=27), the generated results for T4 and cortisol ranged from 17·5 to 39·9 and from 38·0 to 209·0 nmol/L, respectively. The calculated reference interval

Table 1. Precision values (CV, %) for the measurement of T4 and cortisol using the AIA-360• analyser

Analyte	Low CV (c/f)	Medium CV (c/f)	High CV (c/f)	Average CV (c/f)
T4 within-run	8·4/4·1	5·8/5·6	5·3/3·3	6·5/4·3
T4 between-run	9·1/11·2	5·5/4·2	6·3/4·0	7·0/6·5
Cortisol within-run	2·3/4·7	3·6/6·2	2·0/3·0	2·6/4·6
Cortisol between-run	2·9/8·2	2·5/1·8	6·7/4·0	4·0/4·7

CV Coefficient of variation, T4 Thyroxine, c/f Canine/feline
Low/medium/high: samples containing relatively low, medium or high concentrations of analyte

Table 2. Median, range values and Deming's regression analysis results for cortisol (nmol/L) and total T4 (nmol/L) measured in 60 canine and 60 feline samples using the AIA-360• and Immulite• analysers

Analyte	AIA-360 median	Range	Immulite median	Range	Slope estimate	95% CI	Intercept estimate	95% CI	r_s
T4 (dogs)	24·2	<6·4 to 131·0	17·8	<6·4 to 140·0	0·93	0·88 to 0·98	4·8	3·3 to 6·3	0·94
Cortisol (dogs)	294·6	<28·0 to 787·0	327·0	<28·0 to 1059·0	0·79	0·74 to 0·85	26·5	6·3 to 46·8	0·97
T4 (cats)	31·4	<6·4 to 321·0	28·3	<12·9 to 363·0	0·86	0·83 to 0·89	4·9	1·9 to 8·0	0·97
Cortisol (cats)	74·6	<28·0 to 286·0	65·4	<28·0 to 372·0	0·81	0·76 to 0·86	21·3	13·7 to 29·0	0·97

CI Confidence interval, T4 Thyroxine

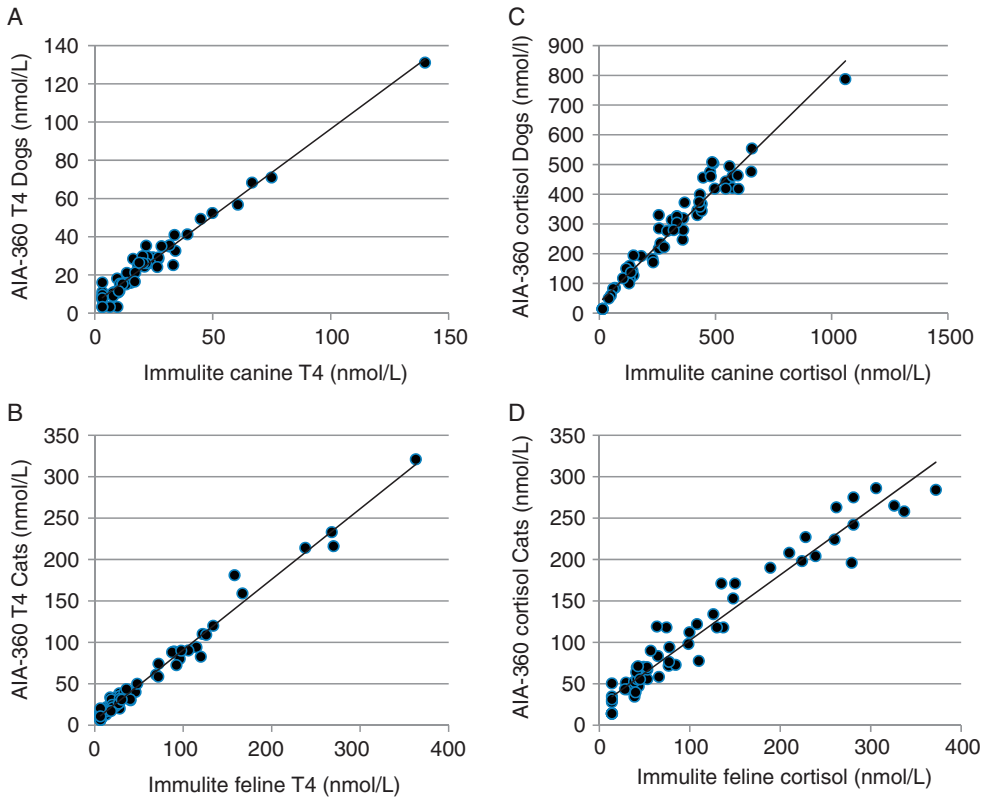

FIG 1. Deming regression plots of Immulite versus AIA-360 analysers for canine T4 (A), feline T4 (B), canine cortisol (C) and feline cortisol (D). The solid lines represent the best-fitted lines

Table 3. Data from Bland–Altman difference plots used to determine the agreement between the AIA-360• and Immulite• analysers

Analyte	Bias	95% CI for the bias	Number of outliers
T4 (dogs)	3·2	–5·5 to 11·9	6/60
Cortisol (dogs)	–40	–163 to 83	3/60
T4 (cats)	–3·5	–31·2 to 24·2	4/60
Cortisol (cats)	0·6	–55·2 to 56·3	4/60

CI Confidence interval, T4 Thyroxine

(mean ±2 sd) for T4 was 13·0 to 45·0 and for cortisol was less than 28·0 to 223·0 nmol/L.

DISCUSSION

To the authors' knowledge, this is the first study in which the AIA-360 and Immulite analysers have been used simultaneously for the measurement of T4 and cortisol in dogs and cats.

The precision studies using the AIA-360 analyser revealed CVs that ranged, depending on the species and hormone concentration, from 1·8 to 11·2% with an overall average CV of ≤7%. These CVs indicate good precision as they are markedly lower than 15%, the maximum acceptable CV according to the industry's bioanalytical guidelines (US Department of Health and Human Services, FDA, CDER, CVM 2001) and lower than the CV previously reported for measuring cortisol by chemiluminescence (CV ≤20%) (Singh *et al.* 1997). In addition, the CVs for T4 using the AIA-360 (range: 3·3 to 11·2%) were similar to those (range: 5 to 9%) reported for the measurement of T4 in dogs and cats with the Immulite analyser (Kemppainen & Birchfield 2006).

The correlations between the two analysers for T4 were excellent [r_s=0·94 (canine) and 0·97 (feline)] and similar to those reported in other studies (r_s=0·91, 0·95, 0·97) where the results obtained by the Immulite were compared to those generated by various human assays (Singh *et al.* 1997, Kemppainen & Birchfield 2006).

In dogs, examination of the Bland–Altman data for T4 identified a small positive bias and the presence of six outliers. In three of these outliers the $T4_{AIA}$ results were lower (131·1 *versus* 140·0, 25·1 *versus* 33·8 and <6·4 *versus* 9·6 nmol/L) than the $T4_{Immulite}$ but as all results were similarly above, within or below the Immulite (10·0 to 40·0 nmol/L) and AIA-360 reference intervals (manufacturer: 13·0 to 52·0; this study: 12·0 to 45·0 nmol/L), it was concluded that the degree of disagreement between the two instruments would not affect clinical decision making in these cases. In the other three outliers the $T4_{AIA}$ results were higher (35·3 *versus* 21·8, 28·4 *versus* 16·3 and 16·0 *versus* <6·4 nmol/L) than those obtained by the Immulite. In one of these cases this difference (16·0 *versus* <6·4 nmol/L) could have had a clinically significant impact as the T4 was below the Immulite but within both AIA-360 reference intervals.

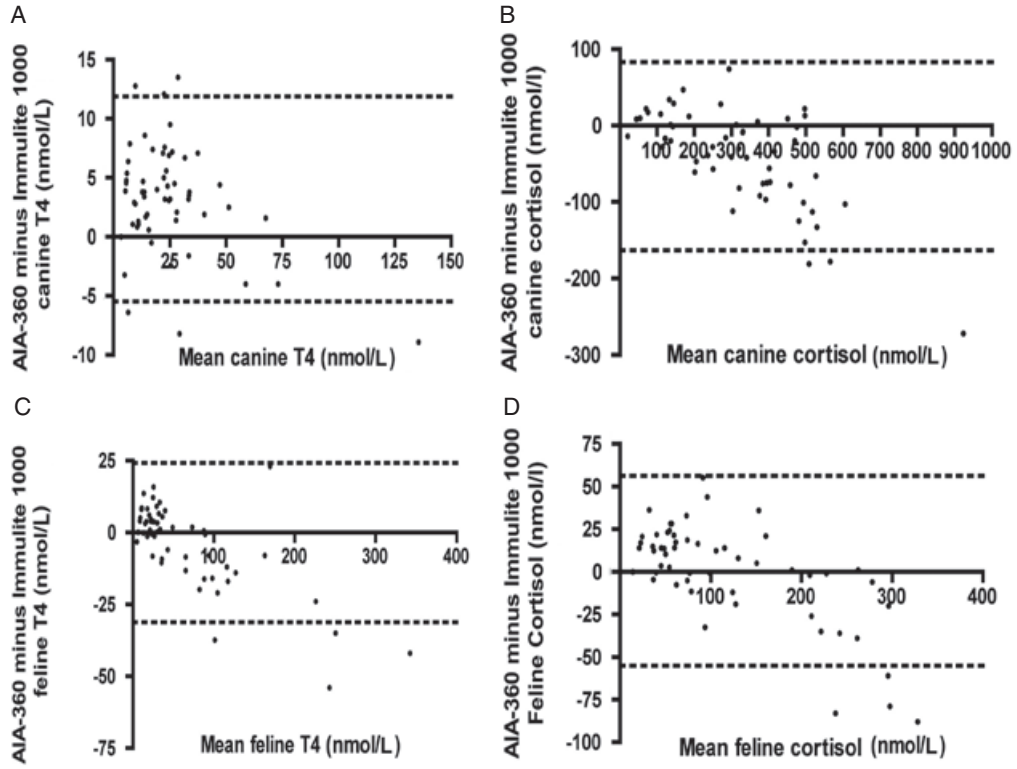

FIG 2. Bland–Altman difference plots using the AIA-360 and Immulite analysers for canine T4 (A), canine cortisol (B), feline T4 (C) and feline cortisol (D). The dashed lines indicate the limits of agreement (mean of the differences ±2 sd)

Examination of the data revealed that in 51 of the 60 samples (85%) the $T4_{AIA}$ values were higher than those obtained with the Immulite. In only one sample the disagreement between the two instruments ($T4_{AIA}$=18·1, $T4_{Immulite}$=9·5 nmol/L) could have affected the clinical decision making. Unfortunately, none of these samples were reanalysed and therefore an operator and/or instrument error cannot be ruled out. In 5 of the 60 samples (8%), the $T4_{Immulite}$ results were above the reference interval (140·0, 66·7, 60·7, 49·9 and 44·9 nmol/L) while the same samples also generated results (131·1, 68·3, 56·7, 52·4 and 49·3 nmol/L) above both AIA-360 reference intervals. In addition, there were no cases with T4 concentrations above the AIA-360 but within the Immulite reference intervals.

In contrast to the findings of the canine data, the feline difference plots for T4 identified a small negative bias. This observation was confirmed as examination of the raw data revealed that in 30 of the 60 samples (50%) the AIA-360 generated values lower (median: 58·6 nmol/L) than the Immulite (median: 69·4 nmol/L), which included the four outliers (321·0 *versus* 363·0, 216·1 *versus* 270·3, 233·2 *versus* 268·0 and 82·6 *versus* 120·1 nmol/L). However, because all outliers were above the Immulite (15·0 to 60·0 nmol/L) and both AIA-360 reference intervals (manufacturer: 10·0 to 55·0; this study: 13·0 to 45·0 nmol/L) it was concluded that the degree of disagreement between the $T4_{AIA}$ and $T4_{Immulite}$ values would not have affected the clinical decision making in these cases and therefore was considered irrelevant. The Immulite generated results above the reference interval in 20 cases (range: 69·4 to 363·0 nmol/L) while the same cases also generated results (range: 58·6 to 321·0 nmol/L) above the AIA-360 reference intervals. In addition, there were no cases with T4 concentrations above the AIA-360 but within the Immulite reference intervals. On the basis of these findings it is suggested that the AIA-360 analyser can be used for the diagnosis of feline hyperthyroidism. However, it should be mentioned that T4 concentrations within the reference interval cannot rule out hyperthyroidism as some hyperthyroid cats with concurrent nonthyroidal disease and/or early or mild hyperthyroidism may generate results within the reference interval (Peterson *et al.* 2001).

The AIA-360 employs the same assay for measuring T4 in dogs and cats, while in this study the Immulite used a different immunoassay for each species. This difference may explain why there was an overall positive bias in dogs but a negative bias in cats. However, irrespective of the Immulite assay, examination of the canine and feline difference plots revealed the presence of a trend for the $T4_{AIA}$ to be lower than the $T4_{Immulite}$ results and for this difference to become more marked as the T4 concentrations increased in the samples. It is suggested that because there were more feline (n=20) than canine (n=5) samples with T4 concentrations above the reference intervals, the difference between the $T4_{AIA}$ and $T4_{Immulite}$ values was more marked in the feline results leading to the overall negative bias identified in the feline cases.

The correlations between the two analysers for cortisol were excellent for both canine and feline samples (r_s=0·97 and 0·97) and similar to those reported in other studies (r_s=0·91, 0·97 and 0·98) where the results obtained by the Immulite were compared to those generated by various assays (Singh *et al.* 1997, Russell

et al. 2007). In addition, the correlations in this study were similar to that reported in a study (r=0·98) comparing the AIA-360 analyser to a chemiluminescence immunoassay using samples from human patients (Mengozzi *et al.* 2007).

In dogs, the Bland–Altman plots for cortisol identified a negative bias, which increased progressively as the cortisol concentrations increased in serum samples. This observation was confirmed by examination of the raw data which showed that in 38 of the 60 samples (63%) the AIA-360 generated values lower (median: 479·0, range: 100·0 to 787·0 nmol/L) than the Immulite (median: 646·0, range: 128·0 to 1059·0 nmol/L), which included the three outliers (787·0 *versus* 1059·0, 476·0 *versus* 654·0 and 418·0 *versus* 599·0 nmol/L). These outliers were generated from post-adrenocorticotropic hormone (ACTH) serum samples and although all values were above the post-ACTH cortisol reference interval established in the authors' laboratory for the Immulite (90·0 to 475·0 nmol/L), their clinical significance cannot be evaluated without the availability of an AIA-360 reference interval post-ACTH. In 5 of the 60 cases, both the Immulite and AIA-360 generated the same cortisol result of less than 28·0 nmol/L and there were no other samples that generated cortisol results below the Immulite (30·0 to 215·0 nmol/L) and/or AIA-360 (<28·0 to 186·0 nmol/L) reference intervals. Two of these five low cortisol concentrations were measured in post-ACTH serum samples that had been collected from dogs with a final diagnosis of hypoadrenocorticism. On the basis of the above findings and the precision results for samples with low cortisol concentrations (CV <3%) it is proposed that the AIA-360 can be used for the investigation of cases where hypoadrenocorticism is suspected.

In cats, the difference plots for cortisol identified a very small and insignificant positive bias for the measurement of cortisol. This was because the number of samples (53%; 32 of 60) with the AIA-360 generating higher cortisol results than the Immulite was very similar to the number of samples (47%) with the AIA-360 generating values lower than (n=22) or equal (n=6) to the Immulite. However, examination of the data revealed that as the cortisol concentrations increased there was a trend for the AIA-360 results to be lower than those of the Immulite leading to four outliers (284·0 *versus* 372·0, 265·0 *versus* 326·0, 258·0 *versus* 337·0 and 196·0 *versus* 279·0 nmol/L). Although all outliers were above the Immulite reference interval (<28·0 to 200·0 nmol/L), three were above the reference interval established in this study for the AIA-360 (<28·0 to 223·0 nmol/L) and only one was above the manufacturer's reference interval (30·0 to 270·0 nmol/L). Even so, the clinical significance of these differences is questionable, taking into account the limited diagnostic value of basal cortisol measurement in feline patients.

Examination of the canine and feline data identified a trend for the $cortisol_{AIA}$ to be lower than the $cortisol_{Immulite}$ results and for this difference to become more marked as the cortisol concentrations progressively increased in the samples. Therefore, it is proposed that because there were more canine (n=51) than feline (n=22) samples with cortisol concentrations higher than 100 nmol/L, the negative bias was more marked in the canine values resulting in the overall negative bias observed in the canine cases.

A limitation of this study is that the number of clinically healthy animals employed for the establishment of reference intervals is lower than the minimum number of 120 individuals, which is recommended by the International Federation of Clinical Chemistry (IFCC) (Geffre *et al.* 2009). Even so, the calculated reference intervals were similar to those provided by the manufacturer of the AIA-360 analyser. None of the 11 samples with mild to moderate haemolysis or lipaemia generated the set of results identified as outliers in the Bland–Altman plots; however, detailed information is not available on the effect of lipaemia on the performance of the AIA-360 analyser. Interference studies should be performed using canine and feline samples to investigate this further.

Conclusions

Following a short training and familiarisation period the AIA-360 is easy to use, provides results quickly and is simple to maintain. The size of the instrument similar to that of an in-house haematology analyser and its wasteless individual test cup technology make it suitable for general practice.

The precision and correlation studies indicate that the AIA-360 is accurate for measuring T4 and cortisol in dogs and cats. However, the agreement studies between the Immulite and AIA-360 indicate that the values generated by the two instruments cannot be used interchangeably and that values should be interpreted using reference intervals established for each individual analyser.

The AIA-360 can be used for the diagnosis of feline hyperthyroidism and for the investigation of cases where canine hypoadrenocorticism is suspected. This study did not evaluate the diagnostic accuracy of the AIA-360 on cases with suspected hyperadrenocorticism. As this condition is diagnosed by an ACTH stimulation test in the presence of appropriate clinical signs it was not possible to prove that the AIA-360 is of use in the diagnosis of this endocrinopathy as post-ACTH $cortisol_{AIA}$ reference intervals are not available. Even so, on the basis of the overall analytical performance of the instrument for the measurement of cortisol, it is suggested that the AIA-360 is likely to be useful in the diagnosis of hyperadrenocorticism. Similarly, in this study, serum thyroid-stimulating hormone (TSH) concentrations were not measured in samples with low T4. As the diagnosis of canine hypothyroidism is most commonly made on the basis of a low T4 in the presence of increased TSH and appropriate clinical signs, it can only be suggested that the AIA-360 is likely to be useful in the diagnosis of hypothyroidism on the basis of the instrument's overall analytical performance for the measurement of T4. More studies designed specifically to evaluate the clinical usefulness of the AIA-360 are needed to confirm these preliminary findings.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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