Urinary Corticoid Concentrations Measured by 5 Different Immunoassays and Gas Chromatography-Mass Spectrometry in Healthy Dogs and Dogs with Hypercortisolism at Home and in the Hospital

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Background: Determination of the urinary corticoid-to-creatinine ratio (UCCR) is an important screening test in the diagnosis of hypercortisolism (HC). However, urinary cortisol metabolites interfere with cortisol measurement in immuno-assays, leading to decreased specificity. Gas chromatography-mass spectrometry (GC-MS) is considered the gold standard for steroid hormone analysis, because it provides a high level of selectivity and accuracy.

Objectives: To prospectively compare the UCCR of healthy dogs and dogs with HC determined by 5 different immunoassays and by GC-MS and to evaluate the influence of veterinary care on UCCR.

Animals: Twenty healthy dogs; 18 dogs with HC.

Methods: Urine was collected in the hospital and again after 6 days at home. Three chemiluminescence immunoassays (Access 2, Beckmann; Immulite 2000, DPC Siemens, with and without trichloromethane extraction) and 2 RIAs (Utrecht in house; Access Beckmann) were used. GC-MS analyses were performed with Agilent 6890N/5973N. Urinary corticoid concentrations were related to urinary creatinine concentrations.

Results: Immunoassay results were significantly higher compared to GC-MS results. Evaluation of bias plots and clinical assessment made on the basis of the assay results of each dog indicated substantial disagreement among the assays. Sensitivity varied from 37.5 to 75% and with selected assays was lower in samples from day 6 compared to day 0. GC-MS was not superior to the immunoassays in discriminating healthy from HC dogs.

Conclusions and Clinical Importance: Considerable variation must be anticipated comparing different urinary cortisol assays. Establishing an assay- and laboratory-specific reference range is critical when using UCCR.

Key words: Hospital stress; Influence; Reference interval; UCCR; Variation.

Hypercortisolism (HC) is a common endocrine disorder in dogs and is the result of excess cortisol secretion by the adrenal cortex. Determination of urinary corticoid (cortisol and its metabolites) excretion is an accepted screening test in the diagnosis of HC, and the urine corticoid-to-creatinine ratio (UCCR) in a random urine sample is expected to be substantially increased in dogs with HC compared to healthy dogs. The diagnostic sensitivity of the test generally is accepted to be high and ranges between 75 and 95%;[1–4] whereas, specificity of the UCCR ranges between 20 and 77%.[1,3,5,6] Increased UCCRs have been reported in dogs with nonadrenal illness and also have been described by van Vonderen et al[7] in healthy dogs undergoing veterinary care or during hospitalization. Based on their study, it has been recommended to

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Abbreviations:

ATH	adrenal-dependent hypercortisolism
GC-MS	gas chromatography-mass spectrometry
HC	hypercortisolism
PDH	pituitary-dependent HC
UC	urinary corticoid
UCCR	urinary corticoid-to-creatinine ratio

collect urine samples at home at least several days after a visit to a veterinary clinic.[8] However, as mentioned above, the authors evaluated UCCR in healthy dogs[7] and, to date, there are no studies evaluating the influence of hospital stress on urinary corticoid excretion in dogs with HC.

In human medicine, it has been shown that the UCCR is significantly influenced by the assay system used to measure the urine cortisol concentration.[9–12] In veterinary medicine as well, it was recently demonstrated that the type of anticortisol antibody significantly influenced the UC in healthy dogs because of cross-reactivity and interference with urinary cortisol metabolites.[13] This can lead to high variations in the UCCR even if determined with the same methodology or assay system, a phenomenon that has not been extensively evaluated because reference intervals still are occasionally arbitrarily chosen from the literature.[14]

Gas and liquid chromatography-mass spectrometry (GC-MS and LC-MS) are considered to be the gold standards for human steroid hormone analysis because

they are antibody-independent and therefore not susceptible to metabolite interference. For this reason, they provide a high level of selectivity and accuracy.[15–17] In 1 study evaluating human patients for Cushing's syndrome, the positive predictive value could be increased to 90% when LC-MS was used for the determination of UC compared to only 43.5% with an immunoassay.[15] To our knowledge, there have been no studies evaluating canine urinary cortisol excretion using mass spectrometry analysis, either in healthy dogs or in dogs with HC.

The objectives of this study were first to evaluate different urinary corticoid immunoassays and a GC-MS method and to compare their clinical performance in healthy dogs and dogs with newly diagnosed HC. Second, we wanted to investigate the influence of hospital care on urinary corticoid excretion measured by the different assays in both populations (healthy dogs and dogs with HC).

Materials and Methods

Animals

Healthy Dogs. Twenty healthy client-owned dogs, including 8 males (3 castrated) and 12 females (10 spayed) with a median body weight of 27.5 kg (range, 10.4–59.2 kg) and a median age of 5.4 years (range, 2.5–11.5 years), were used. They were determined to be healthy on the basis of history and results of physical examination, CBC, serum biochemical profile, and urinalysis including urine culture. None of the dogs had received any medication for at least 8 weeks before inclusion in the study except routine vaccination, deworming, and heartworm prophylaxis.

Dogs with Confirmed Hypercortisolism. Eighteen dogs with hypercortisolism (HC) were included; 8 of them were male (3 castrated) and 10 female (7 spayed). Their median body weight was 10.6 kg (range, 5.2–44.4 kg) and their median age was 10.5 years (range, 7.0–15.0 years).

All dogs underwent a complete hematologic and biochemical evaluation, urinalysis including urinary culture, LDDS test (IV administration of 0.01 mg/kg dexamethasone; blood sampling before, 4 and 8 hours after administration), a measurement of endogenous ACTH, and an ultrasonographic examination of the adrenal glands.

Dogs were included in the study when consistent clinical and laboratory signs of HC were detected, the LDDS yielded a positive result (serum cortisol concentration 8 hours after dexamethasone administration >1.0 µg/dL),[18–20] and treatment with trilostane produced an adequate response (starting dosage of 2–5 mg/kg; re-evaluations performed as described previously).[21] Pituitary-dependent HC (PDH) was diagnosed in 17 of the 18 dogs on the basis of the concentration of endogenous ACTH and a bilateral symmetrical appearance of the adrenal glands, with or without enlargement, on ultrasonography. Follow-up period of trilostane treatment ranged from 3 to 40 months (median, 24 months) and clinical signs shown at presentation were well controlled in 16 of the 17 dogs. One dog was euthanized without treatment and diagnosis was confirmed postmortem by histopathologic examination, which showed nodular hyperplasia of the cortex of the adrenal glands and an adenoma of the pituitary gland.

HC caused by adrenocortical tumor (ATH) was suspected in only 1 of the 18 dogs on the basis of a low endogenous ACTH concentration and the finding of an adrenal mass by ultrasonography. Diagnosis was confirmed after adrenalectomy by histo-

logic examination, which showed an adenoma in the cortex of the adrenal gland. This dog had a high-positive result on the LDDS test (basal cortisol 5.5 µg/dL, 4-h cortisol 4.2 µg/dL, 8-h cortisol 4.1 µg/dL), and ACTH stimulation test (basal cortisol 4.3 µg/dL; post-ACTH cortisol, 65 µg/dL) as well as a positive UCCR with all assays. Therefore, it was included in further statistical analysis together with the dogs with PDH.

The study protocol was officially approved by the veterinary office of the canton of Zurich and was in accordance with the guidelines and directives established by the Animal Welfare Act of Switzerland (TVB 199/2004). Informed consent of all pet owners was obtained before including the dog in the study.

Urine Collection. Voided urine samples were collected in the hospital (day 0) immediately after physical examination and blood sampling, but before any further procedures (eg, ultrasonography of the abdomen) were performed. All owners were asked to collect urine at home in the morning 6 days after the hospital visit (day 6). The urine samples were divided into 6 aliquots each, labeled, and stored at −80°C. Urine of day 0 was available from all 20 healthy and all 18 HC dogs; day 6 urine was available from all 20 healthy and 8 HC dogs, whereas in 10 dogs trilostane treatment was started before obtaining the day 6 urine sample.

Analytical Procedures

Urine aliquots were shipped on dry ice to the respective laboratory commercially offering the chosen assay.[a-f] Urine was thawed immediately before analysis. Samples were assayed by use of 5 different immunoassays and a GC-MS method as described below and summarized in Table 1.

Chemiluminescence Immunoassays. The 3 chemiluminescence immunoassays were as follows: Access 2, Beckmann Coulter (assay A); Immulite 2000, DPC Siemens (assay B); Immulite 2000, DPC, but with trichloromethane extraction of the urine before cortisol analysis and measured in a different laboratory (assay C).

Radioimmunoassays. An in-house radioimmunoassay was performed in the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University,

Table 1. Characteristics of the evaluated cortisol assays

	Measuring Device	Cortisol Measurement Method	Reference Interval*
Assay A	Access 2 (Beckmann Coulter)	Chemiluminescence immunoassay	na
Assay B	Immulite 2000 (Siemens)	Chemiluminescence immunoassay	$<5 \times 10^{-6}$
Assay C	Immulite 2000 (Siemens)	Chemiluminescence immunoassay with trichloromethane extraction	$4–10 \times 10^{-6}$
Assay D	In-house assay system	Radioimmunoassay	$<8.3 \times 10^{-6}$
Assay E	RIA Beckmann Kit (Beckmann Coulter Access)	Radioimmunoassay	$7–10 \times 10^{-6}$
Assay F	6890N – 5973N (Agilent Technologies Inc)	GC-MS	na

*Given by the respective laboratory, na: none given for dogs.

Netherlands, previously described by Rijnberk et al[6] (assay D). Assay E was a commercial RIA from Beckmann Coulter Access.

All assays were performed according to the manufacturers' instructions by the respective laboratory (see above).

Gas Chromatography–Mass Spectrometry (GC-MS). Urine sample preparation was performed as follows: to 5 mL of urine, known amounts of deuterated standards were added: 131.7 ng of cortisol-9,11,12,12-d4 and 117.00 ng of cortisone-α-1,2-d2. After the addition of 10 mL of dichloromethane, the mixture was extracted on a rotator for 20 minutes. The samples were centrifuged at 3000 rpm for 5 minutes, the phases were separated, and the organic layer was transferred to a new tube. After evaporation of the solvent, the extracts were derivatized to form the methyloxime-trimethylsilyl ethers. A volume of 100 μL of methoxyamine hydrochloride 2% in pyridine was added and the samples heated at 60°C for 1 hour. After evaporation of the solvent, 100 μL of trimethylsilyl imidazole was added and the extracts derivatized for 16 hours at 100°C. Thereafter, the derivatized samples were purified by gel filtration on Lipidex-5000 columns to remove the excess derivatization agent.

GC-MS analyses were performed with an Agilent 6890N/5973N instrument[g] in the selected ion monitoring (SIM) mode. One characteristic ion was measured for each compound analyzed: m/z 605 for cortisol, m/z 609 for cortisol-d4, m/z 531 for cortisone, and m/z 533 for cortisone-d2.

Urinary Corticoid-to-Creatinine Ratio. Urinary corticoid concentrations were related to urinary creatinine concentrations (Jaffe kinetic method, initial rate reaction) and UCCR calculated and expressed as $\times 10^{-6}$ for all assays.[4] UCCR reference intervals given by the respective laboratory for each assay are presented in Table 1.

Statistical Analyses

Data were analyzed using nonparametric statistical methods.[h,i] Dogs with PDH and the 1 dog with ATH were classified into 1 group labeled HC.

To assess agreement between the GC-MS and the 5 different immunoassays, Spearman rank coefficient of correlation, linear regression analysis by Passing-Bablok, and Bland-Altman difference plots using the Microsoft Excel add-in Analyse-it[j] were performed.[22,23]

The Wilcoxon matched pairs test was used for comparisons between day 0 and 6 with the respective assay, and the Mann-Whitney U-test was used for comparisons between 2 different assays. Values of $P < .05$ were considered statistically significant.

Results

Agreement between Immunoassay and GC-MS Results (Healthy and HC Dogs)

Results obtained from the 5 immunoassays were analyzed for agreement with the GC-MS results and Bland-Altman difference plots were constructed (Figs 1 and 2).
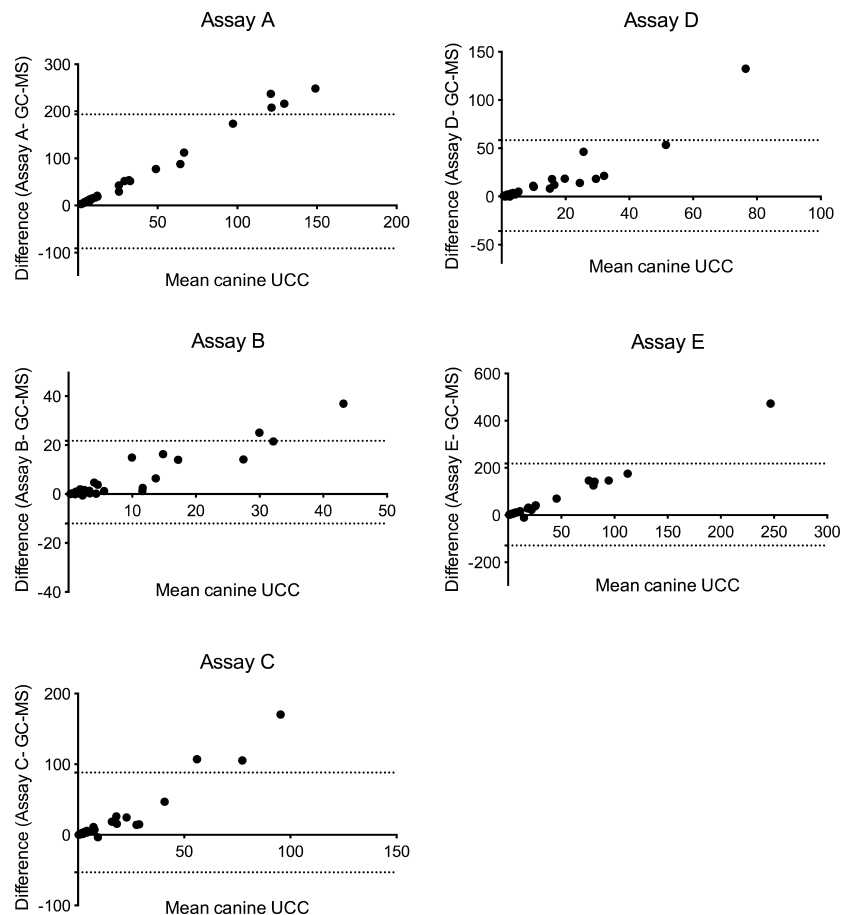

Fig 1. Bland-Altman difference plots comparing results from immunoassays A–E with GC-MS from day 0. The black line is the line of identity. The dashed lines indicate the limits of agreement.
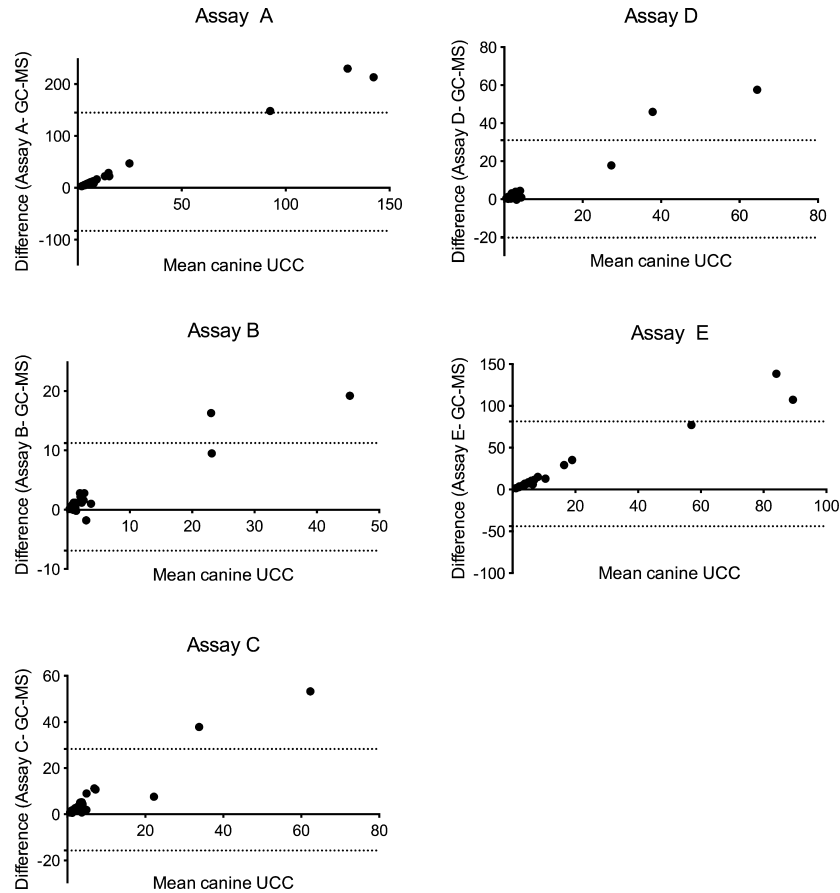
Fig 2. Same as Figure 1, but results from day 6.

Coefficients of correlation among the different immuno-assays and GC-MS from days 0 and 6 are summarized in Table 2a and b. UCCR values obtained with the immunoassays were significantly higher than those obtained with GC-MS on both days ($P < .005$), except for assay B on day 0 ($P = .11$). Bland-Altman difference plots showed positive mean biases for all 5 immunoassays compared to GC-MS indicating a proportional systematic error. On day 0 and day 6, assay B showed only a very small mean bias indicating very good agreement with GC-MS, whereas assay A and assay E showed the highest degree of disagreement compared to GC-MS with high mean biases and also wide 95% limits of agreement reflecting high random error.

Correlation among the Chemiluminescence Immunoassays (Assays A, B, C for Healthy and HC Dogs)

On day 0, the coefficient of correlation between assay A and B was $R = 0.87$, between assay A and C was $R = 0.93$, and between assay B and C was

Table 2. Agreement between the different immunoassays A–E with GC-MS; R: Spearman coefficient of correlation, 6 days after the hospital visit

Assay	R	Intercept 95% CI	Slope 95% CI	Bias 95% Limits of Agreement	Number of Samples n
(a) At the hospital on day 0					
A	0.88	−1.29 (−4.91 to 2.33)	11.30 (9.71–12.96)	51.23 (−91.03 to 193.49)	38
B	0.94	−0.45 (−0.97 to 0.01)	1.95 (1.53–2.46)	4.84 (−12.04 to 21.72)	38
C	0.89	−0.56 (−2.27 to 0.55)	3.72 (3.07–5.30)	17.52 (−53.14 to 88.19)	38
D	0.91	−0.13 (−1.02 to 0.43)	2.81 (2.11–3.23)	11.26 (−35.81 to 58.35)	38
E	0.87	−1.31 (−3.58 to −0.40)	8.19 (7.71–9.82)	44.69 (−128.69 to 218.07)	38
(b) At home					
A	0.78	0.36 (−4.10 to 3.48)	12.84 (8.98–17.55)	30.92 (−82.97 to 144.80)	28
B	0.67	0.07 (−0.47 to 0.23)	1.57 (1.46–2.17)	2.15 (−6.94 to 11.24)	28
C	0.75	0.14 (−2.18 to 1.06)	3.57 (2.74–6.93)	6.27 (−15.74 to 28.28)	28
D	0.73	0.08 (−0.90 to 0.54)	2.63 (2.00–3.95)	5.38 (−20.24 to 31.00)	28
E	0.73	−1.38 (−9.41 to 2.05)	10.38 (5.70–20.55)	18.73 (−43.95 to 81.41)	28

$R = 0.92$. However, the results of assay A were significantly higher than those of assay B ($P < .0001$) and C ($P = .003$). Also, the results of assay C were significantly higher than those of assay B ($P = .01$) (Fig 3A).

On day 6, the coefficient of correlation between assay A and B was $R = 0.64$, between assay A and C was $R = 0.9$, and between assay B and C was $R = 0.6$. The results of assay A were significantly higher than those obtained with assay B ($P < .0001$) and C ($P < .0001$). Also, the results of assay C were significantly higher than those of assay B ($P = .0003$); Figure 3B.

Correlation between Radioimmunoassays (Assays D and E for Healthy and HC Dogs)

On day 0, the coefficient of correlation between assay D and E was $R = 0.86$. Results obtained with assay E were significantly higher than those of assay D ($P = .001$); Figure 3A.

On day 6, correlation between assay D and E was $R = 0.75$. Again, results of assay E were significantly higher than those obtained with assay D ($P < .0001$); Figure 3B.

UCCR Comparison between Day 0 and 6 in Healthy Dogs

There was no significant difference in UCCRs between day 0 and 6 in any of the assays. Comparing single UCCRs of day 0 and day 6 with the upper limit of the reference range given by the respective laboratory identified false-positive results in 1, 1, 0, and 8 dogs on day 0 compared to 0, 0, 0, and 4 dogs on day 6 with assays B, C, D, and E, respectively. Three of the 4 dogs on day 6 with false-positive UCCR were the same that were false positive on day 0 with assay E. Because no upper limit of reference was given for assay A and GC-MS, the number of positive results was not calculated for these 2 assays.

Median, range, and 95th percentiles of UCCR values for assays A to E and GC-MS of day 0 and 6 were calculated for all 20 dogs and are given in Tables 3a and b, respectively. The 95th percentile was approximately half of the upper limit of the reference range provided by the respective laboratory with assay D on day 0 and day 6 (Table 3).
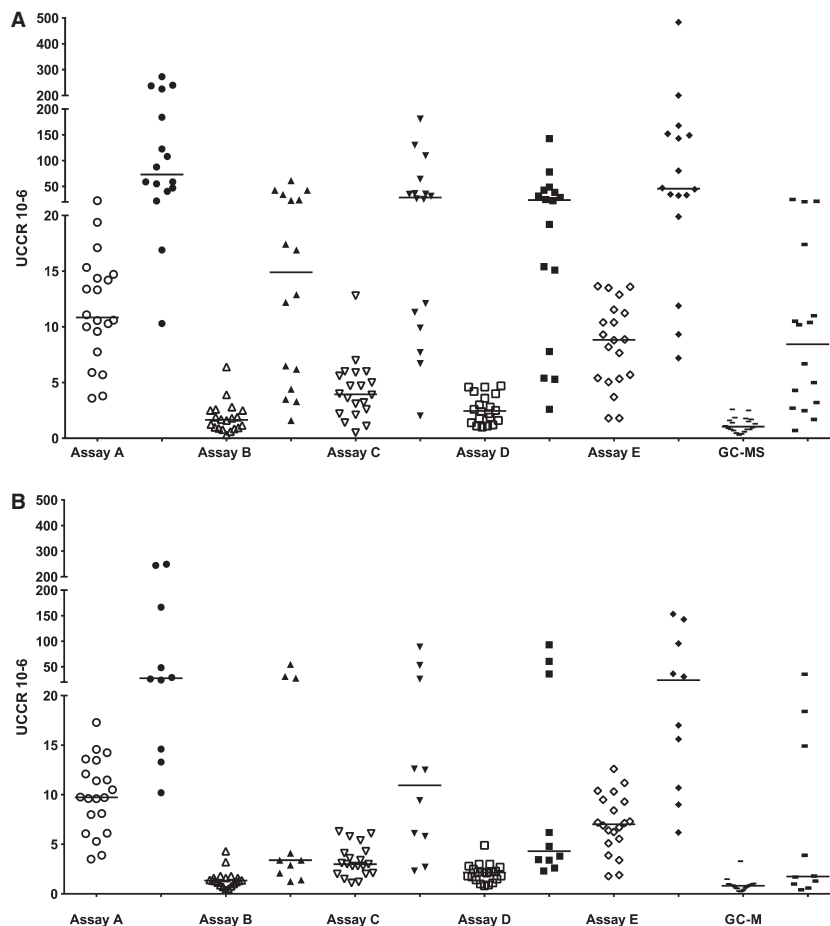

Fig 3. Scatter plot representing UCCRs of healthy dogs (open symbols) and dogs with HC (closed symbols) determined by assays A–E and GC-MS. Median values are indicated by horizontal lines. (**A**) day 0 (hospital); (**B**) day 6 (6 days after hospital visit). Statistically significantly differences are given in the text (Results).

Table 3. UCCRs (median, range, 95th percentile) of 20 healthy dogs obtained by assays A–E and GC-MS, on day 0 (a) and on day 6 (b).

	Assay A	Assay B	Assay C	Assay D	Assay E	GC-MS
(a) Day 0						
Minimum	3.6	0.3	0.5	1.0	1.8	0.3
Maximum	22.4	6.4	12.8	4.7	13.7	2.6
Median	10.8	1.7	3.9	2.5	8.8	1.0
95th percentile	22.2	6.3	12.5	4.7	13.6	2.6
Reference interval*	na	$<5 \times 10^{-6}$	$4{-}10 \times 10^{-6}$	$<8.3 \times 10^{-6}$	$7{-}10 \times 10^{-6}$	na
(b) Day 6						
Minimum	3.5	0.4	1.1	0.8	1.8	0.3
Maximum	17.3	4.3	6.3	4.9	12.6	3.3
Median	9.7	1.3	3.0	2.2	7.0	0.8
95th percentile	17.2	4.2	6.3	4.8	12.5	3.2
Reference interval*	na	$<5 \times 10^{-6}$	$4{-}10 \times 10^{-6}$	$<8.3 \times 10^{-6}$	$7{-}10 \times 10^{-6}$	na

*Given by the respective laboratory, na: none given for dogs.

UCCR Comparison between Day 0 and 6 in Dogs with HC

For comparison of day 0 with day 6, only 8 dogs were available because in 10 of the dogs with HC, trilostane treatment had been started before the day 6 urine sample could be collected.

There was no significant difference between days 0 and 6 with assays A, B, D, E, and GC-MS; but with assay C the results of day 6 were significantly lower than those of day 0 ($P = .01$).

Comparing single UCCRs of day 0 and day 6 with the reference range given by the respective laboratory identified false-negative results in 2, 2, and 3 dogs on day 0 with assays B, C, and D, respectively, compared to 5, 4, and 5 with assays B, C, and D on day 6 (Table 4). Because no reference values were given by the laboratory for assay A and GC-MS, UCCRs were compared with the 95th percentile calculated based on the results obtained from the healthy dogs. This analysis identified false-negative results on days 0 in 3 and 3 dogs with assay A and GC-MS, respectively, compared

to 3 and 5 dogs with assay A and GC-MS on day 6 (Table 4). With assay E, there was no difference in numbers of false-negative results on day 0 compared to day 6, with 2 dogs on each day (Table 4).

Discussion

In this study, the UCCR of healthy dogs and of dogs with HC obtained by 5 different immunoassays and by a GC-MS method were evaluated. Except for the in-house radioimmunoassay, which is only performed in 1 institution in the Netherlands,[6] all other immunoassays are offered by accredited European laboratories and 1 of the assays (Chemiluminescence, Immulite 2000) currently is regarded as the most popular assay in veterinary medicine.[24] Therefore, data of this study are of importance for practicing veterinarians. Although results obtained with the immunoassays correlated significantly, evaluation of bias plots and clinical assessment made on the basis of the assay results of each individual dog identified substantial disagreement. Users of GC-MS should anticipate significantly lower UCCRs compared with values obtained by immunologic methods, a fact that is already known from human medicine.[17,25,26] A reason for this systematic overestimation of UC with immunoassays compared to MS is the interference of the antibodies used in immunoassays which can cross-react with urinary cortisol metabolites, such as dihydrocortisol (5 alpha and 5 beta) and tetrahydrocortisol and other related substances, which are high compared with cortisol.[10,27,28] The degree of interference is highly dependent on the antibody that is used. This was shown in a recent veterinary study comparing 2 different antibodies, 1 specific (anticortisol 21-HS antibody) and the other nonspecific (anticortisol-3 CMO antibody). The UCCR of healthy dogs was found to be 8 times higher using the nonspecific antibody.[13] We also observed this difference in our study. Our comparison of 3 chemiluminescence immunoassays determined that although their correlation was good, the UCCRs were significantly higher with assays A and C compared to assay

Table 4. Number of dogs with UCCRs above the upper limit of the reference range given by the corresponding laboratory for assays B, C, D, and E and the 95th percentile calculated from the healthy dogs for assay A and GC-MS compared with numbers of totally tested dogs.

	Day 0		Day 6	
Assay	Positive Identified Dogs/Total Tested Dogs	Percentage	Positive Identified Dogs/Total Tested Dogs	Percentage
A	5/8	62.5	5/8	62.5
B	6/8	75	3/8	37.5
C	6/8	75	4/8	50
D	5/8	62.5	3/8	37.5
E	6/8	75	6/8	75
GC-MS	5/8	62.5	3/8	37.5

B. This also was shown when UCCRs obtained from the 2 RIAs were compared. The UCCRs from assay E were significantly higher than those from assay D. Interestingly, there was an excellent correlation ($R = 0.95$) between assay B (chemiluminescence) and assay D (RIA) (data not shown), and the 2 immunoassays produced UCCRs lower than all other assays, except GC-MS. The 2 assays showed the best correlation with GC-MS, the method recognized as most specific for the determination of cortisol. The antibody used in assay D is known to be the above-mentioned specific anticortisol 21-HS antibody. Based on our results, one might assume that this antibody also is used in assay B. Correlation also was very good between assays A (chemiluminescence) and E (RIA) ($R = 0.92$), and the UCCRs determined by those 2 assays were considerably higher than values obtained with assays B and D. Interestingly, diagnostic efficacy was not worse with assays A and E. This finding indicates that the use of a highly specific antibody does not necessarily improve the diagnostic performance of an assay, but emphasizes that it is more important to appropriately adapt the reference intervals. Our results support the data shown by Zeugswetter et al[13,24] and clearly reinforce the importance of establishing not only method-dependent, but also assay-dependent reference values because the upper limit of a reference interval can differ severalfold among assays. This is of great importance because a change in the upper limit of the reference interval has a direct and dramatic impact on the sensitivity and specificity of an assay and hence will affect the number of false-negative and false-positive results.

UCCRs obtained by the chemiluminescence immunoassay with extraction of the urine were higher than those without extraction on both days 0 and 6. In human medicine, the extraction of urine with dichloromethane before cortisol measurement is not only time consuming, but also leads to additional variability.[29] Therefore, this additional procedure does not add any benefit, nor does it improve the performance of the test, and hence can be omitted.

The use of GC-MS overcomes the interference problems and related lack of specificity discussed above. However, GC-MS techniques are labor intensive and expensive and consequently not yet widely available. More importantly, our data show that the ability of GC-MS to distinguish healthy dogs from those with HC was not superior to that of the immunoassays. As discussed above, from the present data we can only conclude that an accurate and specific urine cortisol determination such as that obtained by GC-MS does not necessarily improve diagnostic sensitivity; its value in improving specificity was not evaluated in this study. We cannot exclude that GC-MS could be used to further investigate increased UCCRs obtained from immunoassays and to assist in the differentiation of the hitherto assumed "false-positive" immunoassay results.

Diagnostic sensitivity of the assays was rather low compared to that obtained in previous studies. However, cases of HC presented to a referral center do not necessarily reflect those found in the general population. The preselection of dogs, including preferentially early cases of HC with only mild signs, may have led to less clear test results. It has been suggested in a recently published ACVIM consensus statement on the diagnosis of HC that the incidence of mild cases has increased over time and that milder cases will have a lower degree of cortisol hypersecretion.[30] Therefore, cutoff values to interpret diagnostic tests established more than a decade ago may not apply anymore. This also has been our experience, which is why we have adapted the cutoff value of the 8 h cortisol concentration of the LDDS test from 1.4 to 1.0 μg/dL.[18–20] Still, the severity of the clinical signs in our dogs cannot be judged objectively because we did not use a score sheet for the examination, which would be a prerequisite to confirm our assumption that we included more dogs with only mild HC.

Moreover, it has been stated that in individual dogs day-to-day variation can lead to UCCRs within the reference range, whereas measurement on other days could have identified increased UCCRs in the same dog.[31,32] Another point to consider is that age and body weight were not matched between the healthy and the HC dogs, indeed there was a significant difference between the 2 groups (data not shown). This difference could have affected the appropriateness of the healthy group as a reference.

The influence of veterinary hospital care on the UCCRs of healthy dogs was evaluated in an earlier study.[7] The authors found significantly increased UCCRs if urine was taken in the hospital compared to at home. In our study, we found no difference in the UCCRs of healthy dogs using urine taken at home compared to urine obtained during hospitalization with any of the assays. There was no significant difference in UCCRs of dogs with HC in the hospital compared to at home, except for 1 assay. The discrepancy between our results and those of Van Vonderen et al[7] can be attributed first to differences in animal numbers, which were much lower in our study. Second, the procedures dogs in the previous study underwent before urine sampling were more stressful and included vaccination, orthopedic examination, and hospitalization, which was not the case in our study.

In conclusion, evaluation of each assay is important even if the same methodology is used, and reference intervals or decision thresholds must be determined by the laboratory offering the test. When using GC-MS, significantly lower UCCRs compared to results obtained from immunoassays must be anticipated. However, MS did not improve the unexpectedly low observed diagnostic sensitivity and it was not uncommon that UCCRs of dogs with HC were within the provided reference interval. This might be because of the increasing frequency of HC being diagnosed in early stages, as was suggested in the ACVIM consensus statement on the diagnosis of HC.[30]

Footnotes

Acknowledgments

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