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CLINICAL EVALUATION OF A CANINE-OPTIMIZED POINT-OF-CARE CORTISOL ASSAY FOR THE DIAGNOSIS OF HYPERADRENOCORTICISM

Small Animal Internal Medicine

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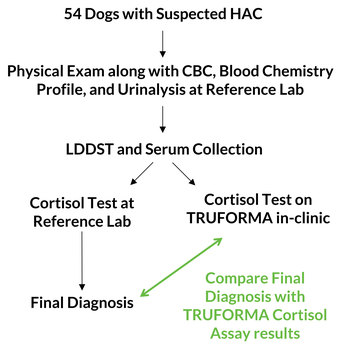
Introduction

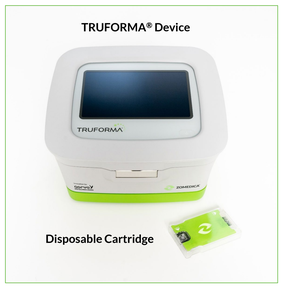
Hyperadrenocorticism (HAC), also known as Cushing's syndrome, is a common endocrine disorder in which excess cortisol is present in the bloodstream. In dogs, this overproduction of cortisol can be due to natural causes like a pituitary gland tumor (pituitary-dependent HAC [PDH], ~85% of cases) or a functional adrenal tumor (FAT, ~15% of cases).[1] Additionally, iatrogenic HAC can be observed in instances where glucocorticoids are over-administered. Middle-aged and geriatric dogs are most commonly diagnosed with HAC and may display a wide array of clinical signs including polydipsia, polyuria, abdominal distension, and lethargy.

The screening test of choice for suspected non-iatrogenic HAC in dogs is a low-dose dexamethasone suppression test (LDDST).[2] For this test, serum is collected before intravenous injection of dexamethasone as well as 4- and 8-hours post-delivery. Serum cortisol concentrations are then determined for each time point and based on how the animal's cortisol levels respond to dexamethasone, a diagnosis, and in certain cases a disease origin, can be discerned. A point-of-care (POC) canine cortisol immunoassay that uses highly sensitive bulk acoustic wave (BAW) technology was developed to provide accurate and precise quantification of canine serum cortisol concentrations across the clinically important range. This study examines the accuracy of this newly developed test for diagnosing dogs with HAC when compared to a diagnosis made using reference laboratory results.

Materials and Methods

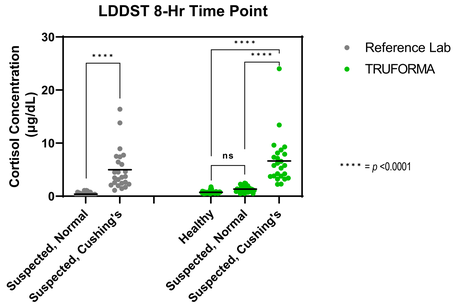
A total of 54 dogs from three veterinary clinics suspected of having HAC underwent a physical exam, standard lab work, and a prescribed LDDST protocol. Serum was collected at baseline in addition to 4- and 8-hours after dexamethasone injection (0.01 mg/kg). Cortisol levels were evaluated at a reference laboratory and in-clinic on TRUFORMA® devices. A diagnosis for each animal was made by an endocrinologist based on clinical signs and reference lab results. The TRUFORMA Cortisol Assay results for each animal were then compared to the endocrinologist's final diagnosis to evaluate overall assay agreement.



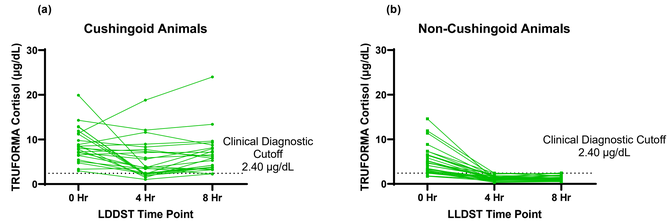


Suspected Cushingoid Cases

The TRUFORMA Cortisol Assay Accurately Diagnoses Cushing's Disease. The serum cortisol concentration of the 8-hour post-dexamethasone administration blood draw is used to determine whether the LDDST supports a diagnosis of HAC.[2] A cortisol value above the assay and/or lab-specific cutoff is indicative of HAC. For the 54 dogs examined in this study, those that were ultimately diagnosed with normal adrenal function and those that were diagnosed as Cushingoid had significantly different 8-hour cortisol concentrations with the TRUFORMA Cortisol Assay, mirroring the cortisol results generated at the reference laboratory.



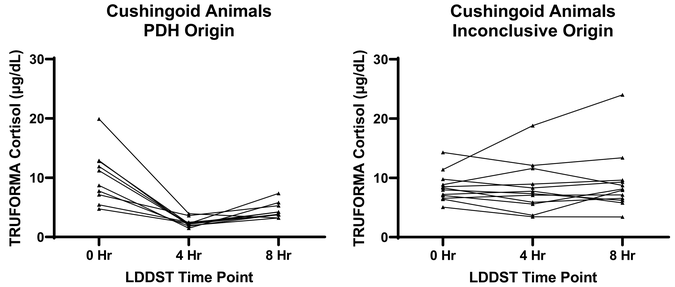
Interestingly, while the TRUFORMA Cortisol Assay successfully differentiated suspected HAC animals into Normal and Cushingoid statuses based on their 8-hour LDDST time point, there was a slight divergence between the 30 Suspected, Normal dogs and a separate population of 40 Healthy animals examined during assay validation (mean cortisol concentrations of 1.33 µg/dL and 0.75 µg/dL, respectively). The 40 Healthy animals were research colony canines that underwent LDDS testing and comprised the data set that led to a TRUFORMA cortisol reference range of ≤1.0 µg/dL for the 8-hour LDDST cortisol reading. However, because these Healthy dogs could not be presenting any clinical signs consistent with HAC for inclusion, the predictive value of this range for evaluating suspected Cushingoid dogs is likely reduced. For that reason, analysis of the clinic-based animal population captured in this study was performed to determine if another indicator value could provide more precise diagnostic interpretations.



Ultimately, a clinical diagnostic cutoff of 2.40 µg/dL was decided to be the optimal decision point for the 8-hour TRUFORMA cortisol results. **(a)** For the 24 dogs diagnosed with Cushing's disease by the endocrinologist, 22 (91.7%) had 8-hour cortisol concentrations above the clinical diagnostic cutoff, thus agreeing with the final HAC diagnosis. **(b)** Furthermore, of the remaining 30 enrolled dogs that were diagnosed as Normal by the endocrinologist, 29 (96.7%) were accurately diagnosed with normal adrenal function based on the TRUFORMA cortisol values.

Dogs whose TRUFORMA cortisol concentrations fall between the upper end of the 8-hour LDDST reference range value (1.0 µg/dL) and this 2.40 µg/dL clinical diagnostic cutoff are in a diagnostic "grey zone". Careful analysis of all clinical signs and lab results is recommended for animals in this region as multiple borderline cases were identified by the endocrinologist. In this study, 2 of 6 (33.3%) grey zone animals that returned for a follow-up appointment 4-8 months after their initial visit underwent another LDDST and had reference lab cortisol results consistent with HAC. This suggests that the grey zone (1.0-2.40 µg/dL) may represent pre-Cushingoid animals and regular monitoring of these animals could aid in early identification of Cushing's disease.

HAC Origin Interpretation



After the 8-hour LDDST time point is found to support an HAC diagnosis, analysis of the whole suppression profile may reveal whether the disease is due to a pituitary tumor (PDH) or if a FAT is also plausible. If the 4-hour time point has a cortisol reading below the assay and/or lab-specific cutoff (escape pattern) or is less than half the baseline reading (partial suppression), PDH is inferred. If there is no suppression at the 4-hour time point (i.e., the cortisol concentration is greater than half the baseline reading), the origin of HAC can't be determined without additional testing such as the measurement of plasma eACTH levels or an abdominal ultrasound.[1] For the 22 animals diagnosed with HAC by the endocrinologist and TRUFORMA Cortisol Assay, 10 cases were classified as PDH by the endocrinologist and the other 12 were inconclusive without further evaluation. The results generated by the TRUFORMA Cortisol Assay agreed with 9/10 (90%) of the PDH cases and 12/12 (100%) of the inconclusive cases for an overall HAC origin agreement of 21/22 (95.4%).

Conclusion

These results suggest that the BAW biosensor technology utilized in this point-of-care cortisol assay allows for clinical differentiation of dogs with normal adrenal function and those with hyperadrenocorticism. Additionally, the quantitative cortisol concentrations are accurate and allow for discernment of the HAC origin with near-identical agreement to current standard-of-care reference laboratory testing methods. Lastly, the employment of a clinical diagnostic cutoff, in addition to a reference range, will aid practitioners in monitoring pre-Cushingoid animals. Further studies are needed to confirm the effectiveness of the clinical grey zone proposed here along with more specific monitoring guidelines for veterinary professionals.

References

1. Gilor C, Graves TK. Interpretation of laboratory tests for canine Cushing's syndrome. *Topics in Companion Animal Medicine*. 2011;26(2):98-108.
2. Behrend EN, Kooistra HS, Nelson R, Reusch CE, Scott-Moncrieff JC. Diagnosis of spontaneous canine hyperadrenocorticism: 2012 ACVIM consensus statement (Small animal). *J Vet Intern Med*. 2013;27(6):1292-1304.

Conflict of Interest

D. Narwold and A. M. Wood are employees of Zomedica Inc., the co-developer of the TRUFORMA platform and assays. C. Ward is a consultant for Zomedica Inc.