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Comparison of the Vcheck and IMMULITE 2000 methods for cortisol measurement in canine serum

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Summary

The aim of this study was to compare canine cortisol results obtained by the Vcheck method with those obtained by the IMMULITE 2000 immunoassay, which had previously been validated for canine serum. The concentration of cortisol in 44 canine serum samples was determined concurrently by the Vcheck and IMMULITE methods, the latter as the reference method. Cortisol values were compared using Pearson's correlation analysis and simple regression analysis. Agreement between the two methods was calculated with a Bland-Altman plot. According to regression analysis and the Bland-Altman plot, the two methods gave comparable results. The results of the Vcheck method were comparable to those of the IMMULITE 2000 reference method, so the Vcheck may be used as an alternative assay to evaluate serum cortisol concentration in dogs for the diagnosis of adrenal disease.

Keywords: cortisol, dog, immunoassay

Hypoadrenocorticism (HA) is an endocrinopathy in dogs, with prevalence ranging from 0.06% to 0.28%. There is an increased risk for HA in Portuguese Water Dogs, Standard Poodles, Bearded Collies, Cairn Terriers and Cocker Spaniels (7). A genetic predisposition for HA also exists in such breeds as Pyrenees dogs, Nova Scotia Duck Tolling Retrievers, Leonbergers and Pomeranians, (3, 10).

It is presumed that primary HA results from a slowly progressing immune-mediated destruction and consecutive atrophy of the adrenal cortex (2, 6). Other rare causes include trauma and infiltrative damage by neoplasia, abscess and granulomatous inflammation (11, 13). In most cases of HA, gradual destruction of all three layers of the adrenal cortex results in an inadequate secretion of mineralocorticoid and glucocorticoid hormones, leading to typical electrolyte imbalances (hyperkalemia, hyponatremia and hypochloremia) (8). Dogs with HA are frequently presented with vague, episodic and nonspecific clinical signs, including anorexia (89%), vomiting (72%), weight loss (42%) and diarrhea (35%) (8).

Canine hyperadrenocorticism (HAC) is an endocrine disease routinely encountered in primary care veterinary practices, with an estimated prevalence of 0.28% (14). The disease is most commonly due to a functional pituitary tumour, although in approximately 15% of cases an adrenal tumour causes excessive circulatory glucocorticoids, which ultimately produce the classical clinical signs in affected dogs (4). Depending on the duration of the disease, these cases show various combinations of polydipsia, polyuria, polyphagia, muscle atrophy, hepatomegaly, lethargy and dermatological changes (5, 9, 18). The disease is generally associated with older age, with an average age at diagnosis in primary care practice of nine years (14). The disease often occurs in older dogs as one of multiple morbidities, including diabetes mellitus, calcium oxalate urolithiasis, hypothyroidism, pancreatitis and hypertension (19).

In dogs, serum cortisol concentration can be useful in the diagnosis of adrenal and pituitary disorders. Interpretation of serum cortisol concentration is crucial in the diagnosis and management of dogs with both hyperadrenocorticism and hypoadrenocorticism, and decision levels for adrenal function testing are well established.

Many studies have been done on the validation of analyzers for cortisol concentration in dogs (15, 17).

The IMMULITE 2000 is an immunoassay analyzer that has been validated for the measurement of serum cortisol concentration in dogs. The assay is considered to be specific and free of interference (16, 17). The Vcheck is a compact, rapid, automated immunoassay analyzer that needs to be calibrated only once every 14 days, optimizing the cost per result. While the IMMULITE 2000 is usually available only in specially equipped laboratories, the Vcheck is cheaper and well suited for routine work. The Vcheck cortisol test is an enzyme-linked fluorescent assay designed for the Vcheck system. The aim of this study was to compare the Vcheck assay with the IMMULITE 200 immunoassay for measurement of canine serum cortisol concentration.

Material and methods

Non-fasting blood samples were obtained from the cephalic vein of 44 canine patients referred to the Clinic for a prophylactic check-up of their health status (group 1), and 15 dogs referred to the University of Life Sciences in Lublin Poland to undergo adrenal function testing for the diagnosis of hyperadrenocorticism (group 2). The dogs of group 1 included 28 males and 16 females of different breeds aged 4 to 12 years, whereas group 2 comprised 9 males and 6 females of different breeds aged 2 to 9 years. The patients were selected based on the serum cortisol concentration to represent the entire working range of the IMMULITE 2000.

Cortisol concentration 60 minutes after an ACTH stimulation test (5 μg/kg Vetoryl Dechra trilostane) was interpreted as follows: a) a value within a 8-18 μg/dl range is a physiologic result, b) a value within a 19-24 μg/dl range is an indeterminate result, c) a value higher than 24 μg/dl suggests either adrenal or pituitary Cushing's syndrome, d) a value lower than 8 μg/dl suggests iatrogenic Cushing's syndrome. Blood samples were collected into Vacuette Tubes. The samples were immediately centrifuged, and the serum was removed. Cortisol concentration was measured with the IMMULITE 2000 (Siemens Healthcare Diagnostics, Deerfield, IL, USA), which uses a solid-phase competitive enzyme-amplified chemiluminescent immunoassay. This assay was used as the reference method in the study. It has a limit of detection of 0.199 μg/dl (5.5 nmol/L) and a calibration (working) range of 0.725-50.02 μg/dl (20-1380 nmol/L) (16, 22). Cortisol concentrations were concurrently determined using the Vcheck assay – an automated test for the quantitative determination of cortisol in canine serum – on the Vcheck analyzer (Vetexpert). Both analyzers were cleaned, calibrated and operated in accordance with the manufacturer's instructions. The basal range of cortisol levels in the blood of normal dogs is approximately 0-8 μg/dl (20).

A paired Student's t-test was used to test for significant differences between cortisol results obtained by the Vcheck analyzer and IMMULITE 2000. Differences were considered statistically significant at $p < 0.05$.

Cortisol values were compared using Pearson's correlation analysis and simple regression analysis. Agreement between the two methods was calculated with a Bland-Altman plot. The Statistica 10.0 PL software was used for the calculations.

Results and discussion

In group 1, he IMMULITE 2000 analyzer showed a correct blood cortisol concentration of the hormone (0.897-7.35 μg/dl, range 0-8 μg/dl) in 40 samples and an elevated one in 4 samples (8.47-18.20 μg/dl, range 0-8 μg/dl). In the same group, the Vcheck analyzer indicated a correct cortisol concentration in 41 samples (0.76-7.97 μg/dl) and an elevated one in three samples (8.19-16.73 μg/dl). For two of the samples in which the IMMULITE 2000 analysis indicated an elevated cortisol concentration (9.52 and 8.47 μg/dl), the results obtained by the Vcheck were within the normal reference range (7.08 and 7.97 μg/dl). For one of the samples in which the Vcheck indicated an elevated cortisol concentration (8.19 μg/dl), the result obtained by IMMULITE 2000 was within the normal physiological range (7.19 μg/dl).

After ACTH stimulation in group 2, the IMMULITE 2000 analyzer showed normal values of cortisol concentration in two samples (17.6 and 17.9 μg/dl), one indeterminate result (22.7 μg/dl) regarding pituitary or adrenal Cushing's syndrome, and a positive result for iatrogenic Cushing's syndrome in twelve samples (< 8 μg/dl).

By analyzing cortisol concentration with the Vcheck analyzer after ACTH stimulation, we obtained two indeterminate results (19.4 and 21.7 μg/dl), one result confirming pituitary or adrenal Cushing's syndrome (24.5 μg/dl), and twelve results positive for iatrogenic Cushing's syndrome (< 8 μg/dl).

The average cortisol concentration for all samples analyzed was 4.69 μg/dl according to the IMMULITE 2000 device and 4.64 μg/dl according to the Vcheck (Tab. 1).

The results of statistical analysis indicate that cortisol concentrations obtained with the Vcheck analyzer and IMMULITE 2000 did not differ significantly ($p = 0.94$, Student's paired t-test). Pearson's correlation analysis shows a very high consistency of the results obtained by the two analyzers ($r = 0.94$) (Fig. 1, Fig. 2, Fig. 3, Fig. 4).

The Vcheck analyzer has good specificity for determining cortisol concentrations in canine serum. In this study, there was a strong correlation ($r = 0.94$) between cortisol concentrations in canine serum measured by

Tab. 1. Comparison of results obtained by the Vcheck and IMMULITE 2000 methods for serum cortisol determination in 44 dogs

Method	Mean	SD	95% CI	Median	Min.	Max.
Vcheck	4.64	5.213549436	1.330318349	2.97	0.53	24.50
IMMULITE 2000	4.69	4.799882195	1.224764708	3.22	0.50	22.70

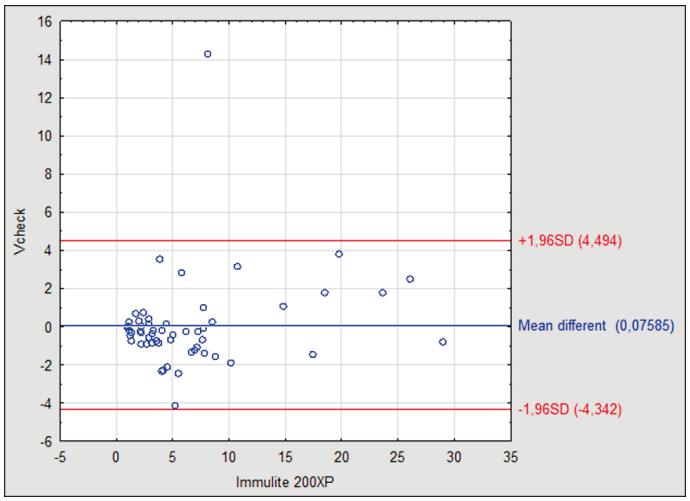

Fig. 1. Comparison between the Vcheck analyzer and the IMMULITE 2000 reference method for cortisol serum concentration (µg/dL) in dogs. Bland-Altman's plot. The solid red lines indicate the mean ± 1.96 SD

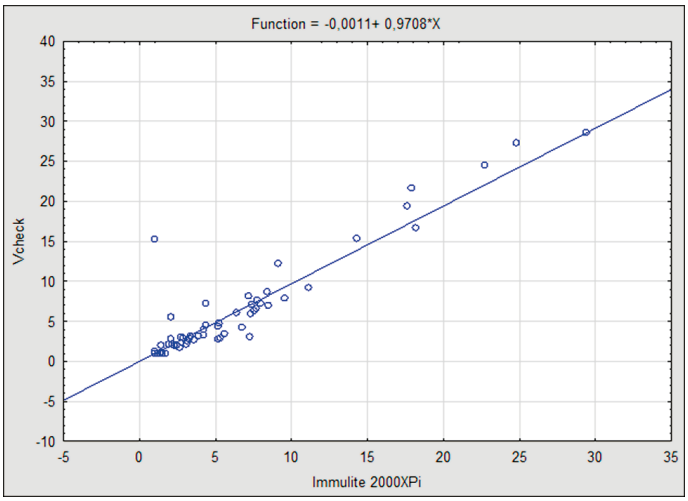

Fig. 2. Comparison between the V-Check analyzer and the IMMULITE 2000 reference method for cortisol serum concentration (µg/dL) in dogs. Passing-Bablock regression. The blue line is the line of identity (y = x)

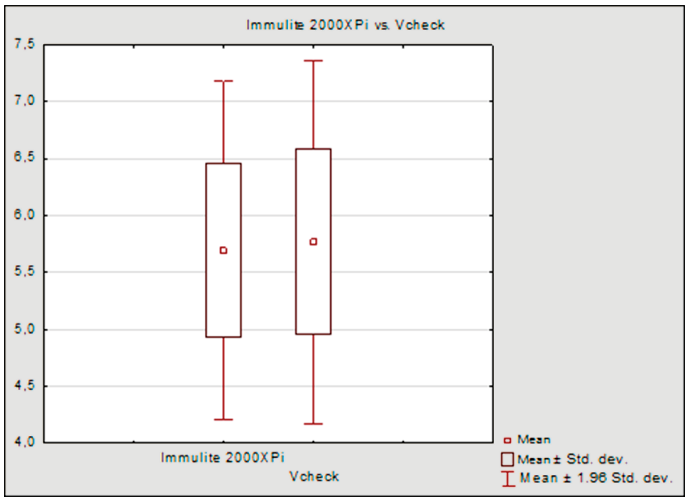

Fig. 3. A paired Student's t-test for significant differences between cortisol results obtained by both analyzers. There was no statistically significant difference between the two analyzers (p = 0.94)

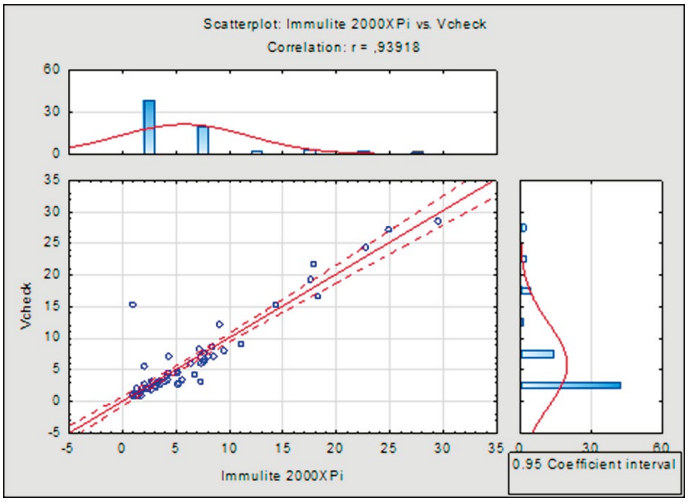

Fig. 4. Pearson's correlation analysis. A very high consistency of the results obtained by the two analyzers (r = 0.94)

the Vcheck and IMMULITE 2000 methods. The tight-fitting regression line demonstrated that the Vcheck had good linearity with the IMMULITE 2000 (Fig. 2). The Bland-Altman test of agreement demonstrated that the Vcheck produced results close to those obtained by the reference method (Fig. 1). Thus, the cortisol concentrations obtained by the Vcheck and IMMULITE 2000 methods were highly comparable in this range of values, which includes the cortisol concentrations obtained after ACTH administration.

The Vcheck analyzer was fast and simple to operate. The rapidity of measurement (20 minutes), the small sample required (50 µl), and the wide working range of the Vcheck method, together with its precision, linearity and comparability to the reference method, make it suitable for canine serum cortisol analysis in samples obtained as part of dynamic endocrine function testing, such as ACTH stimulation tests.

Quick and easy to use analyzers for determining blood parameters such as cortisol concentration in dogs are becoming increasingly attractive for veterinary clinics, not only because of the more commonly performed diagnostics of hypoadrenocorticism and hyperadrenocorticism in dogs in a clinical environment. Proverbio et al. (2009) analyzed the suitability of the VIDAS ELFA human analyzer for determining cortisol concentration in dogs. The VIDAS cortisol test is an enzyme-linked fluorescent assay (ELFA) designed for the MiniVidas system. The MiniVidas has been successfully used to measure the concentration of several human hormones, including insulin, human chorionic gonadotropin, progesterone and cortisol (1, 12). The operating principle of this analyzer is similar to that of Vcheck, and the results obtained by the authors were, like Vcheck results, highly comparable to IMMULITE 2000 results.

Thus, our observations and literature data indicate that rapid analyzers are good for the clinical assessment of adrenal axis function. ”Good” performance means the method is acceptable if used in a highly controlled and carefully monitored instrument system (22). The authors would like to emphasize that each laboratory ought to have a reference interval for cortisol concentration, and caution should be used in determining the cut-off values necessary to differentiate between dogs with and without HAC.

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