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BRIEF COMMUNICATION

Establishment of a reference interval for a novel viscoelastic coagulometer and comparison with thromboelastography in healthy cats

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Background: Viscoelastic analysis provides information on the dynamics and strength of clot formation as well as clot stability. A novel point-of-care viscoelastic test (Viscoelastic Coagulation Monitor Vet, VCM Vet) could be more cost-effective, simpler to use, and more portable than thromboelastography (TEG).

Objectives: The primary aim of this study was to establish a feline reference interval (RI) for the VCM Vet. A secondary aim was to compare VCM Vet analysis with TEG in healthy cats.

Methods: Fifty-six healthy cats were enrolled in this study. Linear regression was completed to determine whether age and CBC parameters were associated with the VCM Vet parameters and if TEG parameters were correlated with VCM Vet data. Statistical significance was set at $P < .05$.

Results: Fifty-three VCM Vet tracings were used to determine RIs for healthy cats. The determined RIs were: clot time (CT) 104-438 seconds; clot formation time (CFT) 104.5-488 seconds; alpha angle (AA) 30.5°-70°; a10 13.8-32.7 VCM units; a20 19.2-40.1 VCM units; maximum clot formation (MCF) 22.5-44.8 VCM units; Lysis Index 30 (Li30) 92.9%-100.9%; and Lysis Index 45 (Li45) 92%-100%. Linear regression identified a strong positive correlation between the CT and R-time measured using the VCM Vet and TEG methods, respectively; no other parameters were correlated.

Conclusions: The use of VCM Vet is feasible in cats, and we determined the first described feline RIs for this test. In general, the VCM Vet data did not correlate with TEG in healthy cats.

KEYWORDS

coagulation, feline, point-of-care, VCM Vet

Viscoelastic devices provide an overall assessment of ex vivo hemostatic function. Analyses incorporate the interaction of all coagulation components, including platelets, red blood cells, fibrin, clotting factors, and thrombin.¹ Analysis of the data from thromboelastography (TEG) or rotational thromboelastometry (ROTEM), a well-established viscoelastic assay, provides information not available through traditional coagulation testing, such as clot formation dynamics and clot strength properties, as well as clot stability and fibrinolysis over time.¹

The clinical application of viscoelastic testing is still limited by such factors as cost, the proximity of the patient to the device, and the technical knowledge and skills required.^{2,3} Currently, TEG is more commonly found in academic centers but is rare in private practice. The Viscoelastic Coagulation Monitor Vet (VCM Vet; Entegriion, Inc) is a novel bench-top viscoelastic device that has been developed for humans, especially for trauma and battlefield applications. The advantages of this device include portability, use of smaller sample

volumes of nonanticoagulated blood, lower purchase prices compared with TEG and ROTEM, and technical simplicity.⁴ The use of this machine has not been validated in small animal patients.

The primary aim of this study was to establish an RI for VCM Vet in healthy cats. A secondary aim was to determine if results obtained with this novel device would correlate directly with a well-established testing method (TEG) in healthy cats. Our primary hypothesis was that multiple VCM Vet parameters would statistically correlate with TEG measurements in healthy cats.

2 | MATERIAL AND METHODS

This prospective study was reviewed and approved by the University of California, Davis Institutional Animal Care and Use Committee on 23 June 2017.

Healthy cats ($n = 56$) from a specific pathogen-free, university-owned colony of domestic shorthaired cats were enrolled in this study. All cats had no current or historical cardiac disease, were not on medication, were not enrolled in other simultaneous studies, and had no abnormalities on complete general physical examination. All cats were group-housed and had access to enrichment activities such as toys and scratching posts. They also had a high degree of daily interaction with humans through petting, grooming, and play.

Five milliliters of whole blood was drawn from 59 cats without sedation using a 22 Gauge needle from the jugular vein or 21 Gauge butterfly catheter from the medial saphenous vein. The obtained blood was separated into three aliquots: EDTA (1.8 mL sample volume per tube; 7.5% K3EDTA); sodium citrate (1.8 mL sample volume per tube; 0.2 mL 3.8% sodium citrate), and nonanticoagulated blood (1.4 mL).

Whole blood (300 μ L) without any added anticoagulants was placed immediately into a testing cartridge before insertion into the point-of-care coagulometer (VCM Vet). This procedure was duplicated on a second device for paired analyses. The mean value for every analyte was calculated from these two measurements on each cat and was used to complete the statistical analyses.

The remaining samples were placed in a thermal container without any cooling material and delivered to the laboratory within 30 minutes of collection. The EDTA tube was used for CBC analyses (Coutler ACT diff; Beckman-Coulter Inc). Citrated blood was used for a single measurement on the TEG within 2 hours from collection. Thromboelastography analysis (ie, waiting time, temperature) was conducted according to our clinical laboratory standardized protocol. After being delivered to the laboratory, samples were maintained at room temperature. One milliliter of citrated blood was mixed with kaolin (cat. no. 6300, Haemonetics Corp). Thromboelastography analysis consisted of pipetting 20 μ L of CaCl_2 first and then adding 340 μ L of citrated-kaolin whole blood into plain test cups. All samples were run until completion of the Lysis 30 (LY30) value.

Cats and their data were excluded from the final analyses if complications were encountered during sample collection (ie, difficulty restraining, bleeding abnormalities at puncture site), platelet counts were below 100 000/ μ L, or HCTs were <30%. For every paired VCM

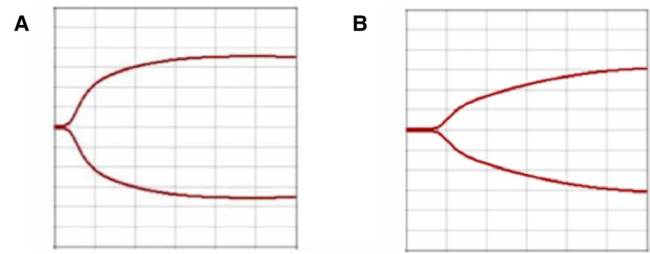


FIGURE 1 Representative viscoelastic tracings from the Viscoelastic Coagulation Monitor Vet (VCM Vet), (A) a normal tracing and (B) discarded tracing on blood from a single healthy cat

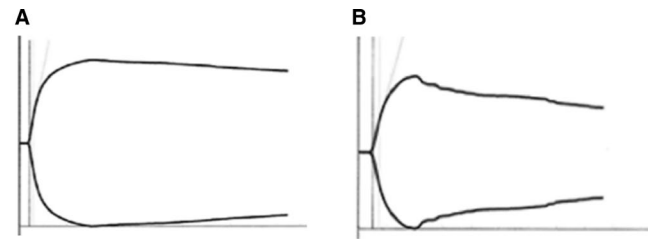


FIGURE 2 Representative viscoelastic tracings from thromboelastography (TEG), (A) a normal tracing and (B) discarded tracing on blood from a single healthy cat

Vet analysis tracings were visually inspected. Both samples were excluded from the final analysis if a marked difference between the two graphical representations was identified. Visual inspection, for sample quality, was also performed on TEG tracings. Samples were excluded if graphical representations were not considered representative of normal physiologic coagulation (eg, clot retraction) (Figures 1 and 2) or if preanalytical error was highly suspected (eg, clot in sample tube, venipuncture problems, irregular sample handling). The correlation between TEG and VCM Vet was calculated on this subpopulation.

Reference intervals (covering 95% of healthy cats) of each analyte were calculated using methods recommended by the American Society for Veterinary Clinical Pathology Quality Assurance and Laboratory Standards Committee.⁵ Data for each analyte were graphed using histograms to check for normality. Data were further checked for normality using the Anderson-Darling test and symmetry using a distribution-free test. If data were not found to be normal or symmetric, the data were transformed using the Box-Cox method and rechecked for normal distribution and symmetry. If either the untransformed or transformed data were normal, then the Robust method was used; otherwise, the nonparametric method was used to determine the reference interval of each analyte. Tukey's test for outliers was then employed, and graphs of the individual data points were examined for outlier values. Outlier values were then removed from the data set if extreme, and the data were reanalyzed without the inclusion of the variables. Where appropriate, data were reported as the median (range) for nonnormal data or mean \pm SD for normal distributions. The precision of our estimates regarding the upper and lower RI limits was further characterized by calculating the 90% CIs for these values. A single sample of whole blood without anticoagulants was run on two separate machines giving replicate

measurements that were used to estimate the within-subject variation based on results from a one-way ANOVA. The within-subject standard deviation estimates the variation attributable to measuring the same sample on different machines.

To look for differences in VCM Vet data between males and females, a *t* test or Mann-Whitney test was used. Linear regression was completed to assess whether age and CBC parameters were associated with differences between cats in the VCM Vet values. Correlations between VCM Vet and TEG data were evaluated based on physiologic processes assessed at similar viscoelastic measurement points. Table 1 Data were graphed and statistical analyses were performed using Reference Value Advisor v2.1 with Microsoft Excel 2010 (Microsoft Corporation) and Stata Version 13 (StataCorp. 2013. Stata Statistical Software: Release 13. StataCorp LP). A *P* ≤ .05 was considered statistically significant.

3 | RESULTS

Blood was obtained from all 56 cats. Six samples were excluded from the final analysis due to major differences in graphical shapes between two simultaneous VCM Vet analysis tracings from the same sample. One curve shape was highly irregular in one cat, and not representative of either a physiologic or pathologic process. In the other two cats, there were markedly abnormal values as well as shape differences between the paired samples. In one cat, the clot time (CT) value was more than 2 SDs below the mean CT value of the retained samples, and in the other cat, the clot formation time (CFT) was greater than 11 times the SD above the mean of the retained sample CFT.

In all of the cases, sample variability was attributed to preanalytical error. No other exclusion criteria were met.

The final analysis included 53 sexually intact (31 females and 22 males), domestic shorthair cats. The median age was 1 year (range 0.5-13). Complete blood count (CBC) parameters for all cats were within normal RIs.

The median CT values for devices 1 and 2 were 295 (101-496) and 304 (81-447) seconds, and the within-subject SD was 57 seconds.

The median CFT values for devices 1 and 2 were 164 (109-477) and 175 (100-499) seconds, respectively; the within-subject SD was 30.8 seconds. The median AA values for devices 1 and 2 were 57° (30°-70°) and 54° (26°-71°), respectively; the within-subject SD was 6.5°. The mean a10 values for devices 1 and 2 were 23.6 ± 10.2 and 22.9 ± 4.7 VCM units; the within-subject SD was 2.5 VCM units. The mean value for a20 was 29.9 ± 5.25 VCM units for device 1, while for device 2, it was 29.3 ± 5.8 VCM units; the within-subject SD was 2.7 VCM units. The mean maximum clot formation (MCF) values for devices 1 and 2 were 33.6 ± 5.8 and 33.7 ± 6.2 VCM units, respectively; the within-subject SD was 2.9 VCM units. The mean LI30 values for devices 1 and 2 were 97.3 ± 1.9% and 96.5 ± 2.2%, respectively; the within-subject SD was 1.0%. The median LI45 values for devices 1 and 2 were 99% (91%-100%) and 100% (92%-100%), respectively; the within-subject SD was 0.7%.

After the exclusion of abnormal tracings, no severe outlier values were identified during the analyses. Reference intervals and measures of central tendency, for each VCM Vet parameter, are presented in Table 2

The mean and SD values for a10 were 20.95 (3.592) and 26.409 (4.133) VCM units for female and male dogs, respectively, and were statistically different (*P* < .001). The mean and SD values for a20 were 27.177 (4.133) and 33.113 (4.522) VCM units for female and male dogs, respectively, and were statistically different (*P* < .001). The mean and SD for MCF were 31.48 (4.55) and 36.73 (5.34) VCM units for female and male dogs, respectively, and were significantly different (*P* < .001). The median (range) for CFT was 187 (130-488) and 142 (104.5-347) seconds for females and males, respectively, and were significantly different (*P* < .001). The mean and SD value for Li30 was 97.84% (1.63%) and 96.24% (1.97%) for female and male dogs, respectively, and were significantly different (*P* = .003).

Weak correlations were identified between several VCM parameters and white blood cell counts. For an increase of 1000 WBCs, a 5 seconds decrease in the CFT ($R^2 = .10$, *P* = .02) was seen; the R^2 value indicated ~10% variation. For an increase of 1000 WBCs, a10 values increased 3.7 VCM units ($R^2 = .13$, *P* = .008); the R^2 value indicated ~13% variation. For an increase of 1000 WBCs, a20 values increased 4.7 VCM units ($R^2 = .17$, *P* = .002); the R^2 value indicated

VCM Vet parameters (units)	TEG parameters (units)	Measurement of	Major influence by
CT (s)	R-Time (min)	Initial fibrin formation	Enzymatic proteases
CFT (s)	K (min)	Speed of clot formation	Fibrinogen, factor XIII, and platelets
AA (degrees)	Alpha angle (degrees)	Speed of clot formation	Factor XIII, platelets, and fibrinogen
a10, a20 (VCM units)	N/A	Clot strength at various time	Factor XIII, platelets, and fibrinogen
MCF (VCM units)	MA (mm)	Maximum clot strength	Platelets and fibrinogen
Li30, Li45 (%)	LY30, LY60 (%)	Fibrinolysis	Plasmin

TABLE 1 Names and parameter comparisons measured with a point-of-care viscoelastic test (VCM Vet) and thromboelastography (TEG) with corresponding units. The physiologic processes and factors primarily affecting (listed in order of importance) the coagulation processes are reported for each parameter

Abbreviations: a10, amplitude at 10 min; a20, amplitude at 20 min; AA, alpha-angle; CFT, clot formation time; CT, clot time; K, kinetics; Li30, Lysis Index at 30 min; Li45, Lysis Index at 45 min; LY30, Lysis at 30 min; LY60, Lysis at 60 min; MA, maximum amplitude; MCF, maximum clot firmness; N/A, not applicable; R-time, reaction time.

TABLE 2 Reference intervals for point-of-care viscoelastic test (VCM Vet) variables and 90% CIs of upper and lower RIs (n = 53 healthy cats)

Analyte	Units	Mean (\pm SD)	Median (range)	RI	90% CI lower	90% CI upper
CT	Seconds	292.2 (76.7)	300.5 (104-438)	103.9-415.5	41.2-160.7	395.7-432.4
CFT	Seconds	186.6 (69.5)	172.5 (104.5-488)	107-344.5	101.6-115.7	300.8-397.0
AA	Degrees	53.4 (8.3)	55.5 (30.5-70)	33.2-66.9	26.9-38.7	64.8-68.9
a10 ^a	VCM units	23.2 (4.7)	23.5 (12-33.5)	13.8-32.7	12.0-15.5	30.7-34.4
a20 ^a	VCM units	29.6 (5.2)	30.5 (18-41)	19.2-40.1	16.9-21.4	38.1-42.3
MCF ^a	VCM units	33.7 (5.5)	34.5 (19.5-45)	22.5-44.8	20.4-24.6	42.5-47.2
Li30 ^a	%	96.9 (2.0)	97 (91-100)	92.9-100.9	92.1-93.7	100.1-101.7
Li45	%	98.7 (2.0)	99.5 (92-100)	92-100	92.0-94.0	100.0-100.0

Abbreviations: a10, amplitude at 10 min; a20, amplitude at 20 min; AA, alpha-angle; CFT, clot formation time; CT, clot time; K, kinetics; Li30, Lysis Index at 30 min; Li45, Lysis Index at 45 min; LY30, Lysis at 30 min; LY60, Lysis at 60 min; MA, maximum amplitude; MCF, maximum clot firmness; N/A, not applicable; R-time, reaction time.

^aNormally distributed data.

~17% variation. For an increase of 1000 WBCs, MCF values increased 0.5 VCM units ($R^2 = .14$, $P = .005$); the R^2 value indicated ~14% variation. Increasing age was negatively correlated with a10 ($R^2 = 0.16$, $P = .003$), a20 ($R^2 = 0.18$, $P = .002$), and MCF ($R^2 = 0.15$, $P = .006$).

From the 53 cats included in the final analysis, 24 TEG data were visually considered acceptable. The excluded data had multiple graphical abnormalities. As per the VCM tracings, abnormalities were present in the graphical representation (eg, clot retraction, hypocoagulability). In all cases, shape abnormalities were attributed to preanalytic errors.

Thromboelastography measurements were mean R-time, 2.46 ± 0.644 minutes, median K 1.2 (0.9-3.8) minutes, median AA 73.55° (59.7° - 76.4°), median MA 60.3 (39.7-69.4) mm, median LY30 5.4% (0%-45.1%). While comparing TEG and VCM parameters, we identified a strong positive correlation between CTs (VCM Vet) and R-times (TEG) ($R^2 = .56$, $P < .001$). A mild positive correlation was identified between MCF and MA ($R^2 = .12$, $P = .05$). There were no significant correlations between the remaining TEG and VCM values.

4 | DISCUSSION

An RI for a novel viscoelastic device (VCM Vet) was described and defined in healthy cats. Cats are predisposed to cardiomyopathies that frequently lead to hypercoagulable states.⁶ A rapidly accessible and cost-effective device capable of assessing hemostatic variables would represent a valuable adjunct in feline clinical medicine. As highlighted by a recently published multicenter veterinary study, there is a growing need to identify monitoring strategies for preclinical cardiomyopathy detection in cats to reduce morbidities and mortalities.⁷ The RIs obtained in our study should only be used as a guideline; as previously recommended by the PROVET group for TEG and ROTEM analyses, it is important that each center create their own "site-specific" reference values.² Viscoelastic technologies are highly sensitive to preanalytic factors, such as sample collection and handling. A standardized protocol should be defined for each center to minimize errors and data variation. Cats included in this study belonged to a specific pathogen-free colony. Although it is challenging to evaluate accurately, a certain

level of inbreeding was expected in the feline population included in this study. For this reason, the population selected might not be entirely representative of a genetic pool of domestic shorthaired cats. According to the American Society for Veterinary Clinical Pathology guidelines, the number of cats included in this study was not ideal but acceptable for the statistical analyses performed.⁵

This study identified strong correlations between CT and R-time only. These parameters identified the contribution of enzymatic proteases to initial clot formation. There was no significant correlation between the remaining VCM Vet and TEG variables. The identification of hypercoagulable states and the evaluation of fibrinolysis were the main clinical indications to perform viscoelastic analyses. Despite the correlations identified in our study, CT and R-time might not represent the most clinically significant parameters, since coagulation factors are conventionally tested via different diagnostic modalities. Each viscoelastic device offers a unique mechanism to measure overall clot formation, and dissolution.² Thromboelastography assesses clot formation in a rotating plastic cylindrical cup with a stationary suspended pin lowered into the center of this cup filled with blood. As the cup oscillates, the pin detects the tension as the liquid becomes more gelatinous through the process of coagulation. This tension/torque is translated into the TEG tracing and standardized measurements.⁸ Viscoelastic Coagulation Monitor Vet measures frictional forces as blood coagulates between two frosted glass surfaces that glide over each other within the cartridge. Once the cartridge is inserted into the device, these frictional forces are measured and transduced by the software and graphically displayed.⁴ Both VCM Vet and TEG generate a qualitative tracing and quantitative values to describe the hemostatic properties of coagulation. Considering the technologic differences between the two tests, it is not entirely surprising to identify a lack of correlation between the measurements reported. Additionally, VCM Vet runs sample analyses on native blood, while TEG was performed after the addition of an activator. This additional difference in blood processing could have further affected correlations between the two devices tested. As previously reported for TEG and ROTEM, the results of one machine cannot be extrapolated to the other.² This study focused on defining the norms for VCM Vet in healthy cats. It

was not designed to determine which device would produce results more representative of the underlying physiologic processes across the spectrum from hypo- to normal to hypercoagulable states. While strong correlations were not detected in cats whose clinical and clotting parameters were all within normal boundaries, higher correlations might well be found if measured across a broader health spectrum. This study confirmed that the use of VCM Vet is feasible in healthy cats. Further studies are required to establish applicability and clinical implications in cats representing a wider health spectrum.

There are some limitations to our study. Cats were considered healthy based on the absence of relevant medical histories and unremarkable physical examinations. None of the cats received echocardiography for subclinical cardiomyopathy at the beginning of the study. Occult cardiomyopathy could have influenced the hemostatic state of some animals. However, cardiomyopathy is less commonly reported in younger cats.⁹ A large number of samples were discarded from the final comparative analyses. Six VCM data sets were not used in the final analyses due to the variations between the tests of two simultaneous samples. Twenty-nine TEGs were also removed from the final analyses due to qualitative and quantitative abnormalities. Samples were similarly transported with care in a thermal container. The reason for the higher TEG variations could have been related to time delays and sample handling problems between venipuncture at the colony site and transport to the laboratory. Temperature fluctuations, transport vibrations, and laboratory time requirements could have all added to these irregular data. The cat colony that was used in this study is located approximately 1 mile from the main laboratory. This study was not designed to validate transportation modalities, but our results are suggestive that TEG analysis in cats is potentially severely impacted by movement and could argue for the importance of using point-of-care devices. We acknowledge that the reduction in sample numbers could have introduced errors in the correlation analyses between TEG and VCM Vet. The exclusion of the markedly deviating curves could have also affected the within-subject standard deviations. Further studies are indicated to evaluate correlations between CBC variations and VCM Vet parameters. As previously reported, HCT variations can significantly affect viscoelastic analyses.¹⁰ In addition, as the cats in this study were healthy with CBC values within the normal RIs, the ability to detect a relationship in cats with other health problems could not be determined from this data. Further studies are needed to ensure the application of VCM Vet in the clinical setting. Studies are currently ongoing for the evaluation of VCM Vet as a therapeutic drug monitoring device.

In conclusion, we defined RIs for a novel viscoelastic device (VCM Vet) in healthy cats. Due to its portable nature, reduced cost, and simpler sample processing method, this device provides numerous advantages over TEG and ROTEM. Even with careful handling in a university veterinary school setting and a distance of only one mile between blood draw and TEG analyses, more than half of the samples had problems sufficient to invalidate TEG results, showing the importance of point-of-collection TEG analysis. We did identify significant correlations between CTs measured using VCM Vet, and R-time measured using TEG. The remaining variables had no significant correlations. VCM Vet analyses could be used to evaluate hemostasis in healthy cats, but the results are not identical to TEG.

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DISCLOSURE

The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

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