Effects of storage time and temperature on thromboelastographic analysis in dogs and horses

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Submitted to the Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, in partial fulfilment of the requirements for the degree MSc

Pretoria, October 2019

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Animal Ethics Committee

PROJECT TITLE	**The effects of storage time and temperature on thromboelastographic analysis in dogs and horses**
PROJECT NUMBER	**V081-18**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. A Lemon**

STUDENT NUMBER (where applicable)	**U_12048722**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES/SAMPLES	**Dogs**	**Horses**
NUMBER OF ANIMALS	**10**	**10**
Approval period to use animals for research/testing purposes		**September 2018 –September 2019**
SUPERVISOR	**Dr. E Hooijberg**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 8 October 2018
CHAIRMAN: UP Animal Ethics Committee	Signature Vinny Bennett

S4285-15

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Acknowledgements

This study was funded by the Clinical Pathology Laboratory at the Faculty of Veterinary Science, University of Pretoria.

My research journey has culminated into what I see as a successful bonfire.

Thank you to Professor Amelia Goddard, my co-supervisor, for providing the initial spark. Your passion for Clinical Pathology is infectious and inspired me to fill out the MSc application. Your input along the way has been invaluable.

Thank you to Professor Emma Hooijberg, my supervisor, for teaching me (from the other side of the world) how to turn a spark into flames. You are the reason we can all sit around a fire and not just a pile of sticks.

Thank you to Haidee Lemon, my mother, for being my constant supporter.

Thank you to Nemo, my cat, for sitting on my lap every step of the way.

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List of abbreviations

aPTT - activated partial thromboplastin time

AT – antithrombin

AVL – Dr Ashleigh V Lemon, principle investigator

CBC – complete blood count

CD – critical difference

CV_A - analytical variability

CV_B - biological variability

CV_G - interindividual variability

CV_I - intraindividual variability

CV_T - total variability

EDTA - Ethylenediaminetetraacetic acid

EHH – Prof Emma H Hooijberg, supervisor

FDPs – fibrin degradation products

FT – refrigeration temperature

MdC – Sr Marizelle de Clerq, veterinary nurse for the Onderstepoort Animal Blood Bank

OABB – Onderstepoort Animal Blood Bank

OTAU – Onderstepoort Teaching Animal Unit

PC – protein C

PE - phosphatidylethanolamine

PolyP – polyphosphate

ProS – protein S

PS - phosphatidylserine

PT – pro-thrombin time

RCV – reference change value

RT – room temperature

rTF – recombinant tissue factor

SOP – standard operating procedure

TAFI - thrombin activatable fibrinolysis inhibitor

TEG – thromboelastography

TF – tissue factor

TFPI - tissue factor pathway inhibitor

tPA – tissue plasminogen activator

TM – thrombomodulin

uPA – urokinase plasminogen activator

vWF – von Willebrand factor

Summary

Thromboelastography (TEG) is an assay that (to date) provides the most extensive analysis of haemostasis. Accessibility of TEG to general practitioners is limited by short sample storage times (30 minutes) and storage temperature (20-23°C). This limited accessibility is evident in human and veterinary medicine, but especially affects veterinary general practitioners in the field.

This study evaluated the stability of canine and equine citrated blood samples when stored for extended periods of time, both at room temperature (RT) (20-23°C) and refrigeration temperature (FT) (2-7.5°C). Citrated whole blood samples from healthy dogs and horses (n=10 for each) were stored for 30 minutes at RT before TEG analysis. Baseline values for TEG variables R, K, α, MA, LY30 and LY60 were compared to those from samples stored for 2 hours, 8 hours, and 22.5 hours, both at RT and FT. Results were compared using ANOVA ($p < 0.05$) and reference intervals.

In dogs, statistically significant differences included shorter R, longer K, decreased MA, and increased LY60 at various time points and storage temperatures from 2 hours. R, K, α and MA values were stable at 8 hours FT. No clinically relevant differences in R, K, or MA values were found at 2 hours RT or FT. In horses, statistically significant difference included shorter R and K, and decreased α, LY30 and LY60 at various time points and storage temperatures from 2 hours. R, K, α and MA values were stable at 2 hours and 8 hours FT. No clinically relevant differences in R, K, or MA values were found at 2 hours RT.

This study suggests that canine samples can be stored for up to 2 hours at RT or FT and equine samples can be stored for up to 2 hours at RT without affecting TEG results.

Chapter 1: Introduction

1.1 Background

Thromboelastography (TEG) is a global assay that analyses haemostasis more extensively (includes cellular and soluble components) than routine coagulation assays (prothrombin time (PT) and activated partial thromboplastin time (aPTT)), providing a continuous detection and recording of viscoelastic changes in whole blood from clot initiation through to lysis. Current limitations on TEG include pre-analytical storage time and temperature, with the current recommendation of 30 minutes storage at room temperature (RT) prior to analysis.[1] This limits the use of TEG to practitioners in a 30 minute radius around facilities equipped with TEG analysers. Increasing the amount of time allowed between blood collection and analysis, as well as extending the range of the allowed storage temperature, will allow wider access for field practitioners to TEG analysis, both in South Africa and globally. To date, there are no published kaolin-activated TEG storage studies in dogs and horses that compare the 30 minutes RT baseline to varied storage conditions. Published TEG storage studies in human and veterinary medicine suggest a (inconsistent) trend towards hypercoagulability over time.

1.2 Objectives

- To determine whether TEG results are affected by time elapsed between collection and analysis, as well as by storage temperature of samples during this time.
- To determine the clinical relevance of any changes observed under the above-mentioned storage conditions.

1.3 Hypotheses

1.3.1 Dogs

H_0: TEG variables will show no statistically significant or clinically relevant differences between samples stored for 30 minutes and samples stored for up to 22.5 hours at RT.

H_1: TEG variables will show statistically significant and/or clinically relevant differences between samples stored for 30 minutes and samples stored for 2 hours, 8 hours and/or 22.5 hours at RT.

H_0: TEG variables will show no statistically significant or clinically relevant differences between samples stored for 30 minutes at RT and samples stored for up to 22.5 hours at refrigeration temperature (FT).

H_1: TEG variables will show statistically significant and/or clinically relevant differences between samples stored for 30 minutes at RT and samples stored for 2 hours, 8 hours and/or 22.5 hours at FT.

1.3.2 Horses

H_0: TEG variables will show no statistically significant or clinically relevant differences between samples stored for 30 minutes and samples stored for up to 22.5 hours at RT.

H_1: TEG variables will show statistically significant and/or clinically relevant differences between samples stored for 30 minutes and samples stored for 2 hours, 8 hours and/or 22.5 hours at RT.

H_0: TEG variables will show no statistically significant or clinically relevant differences between samples stored for 30 minutes at RT and samples stored for up to 22.5 hours at FT.

H_1: TEG variables will show statistically significant and/or clinically relevant differences between samples stored for 30 minutes at RT and samples stored for 2 hours, 8 hours and/or 22.5 hours at FT.

1.4 Benefits arising from this project

- Increasing the amount of time allowed between blood collection and analysis, as well as extending the range of the allowed storage temperature, will allow wider access for field practitioners to TEG analysis, both in South Africa and globally.
- Fulfilment of the requirements of the principal investigator's MSc degree.

Chapter 2: Literature review

2.1 Introduction to haemostasis

Haemostasis is a complex physiological process that balances prevention of blood loss at a site of injury whilst not restricting normal blood flow elsewhere. There are three major steps in haemostasis: primary haemostasis, secondary haemostasis (also known as coagulation) and fibrinolysis. Primary haemostasis involves platelet aggregation and plug formation against damaged endothelium. Coagulation involves activation of coagulation factors and the creation of an insoluble fibrin "clot". Primary haemostasis and coagulation occur concurrently. Fibrinolysis involves clot dissolution once the injury is (hopefully) repaired. The intricacies of haemostasis have been investigated at length, with the development of two widely known models: the cascade model and cell-based model, both of which are discussed in detail below.[2,3]

2.2 The cascade model of coagulation

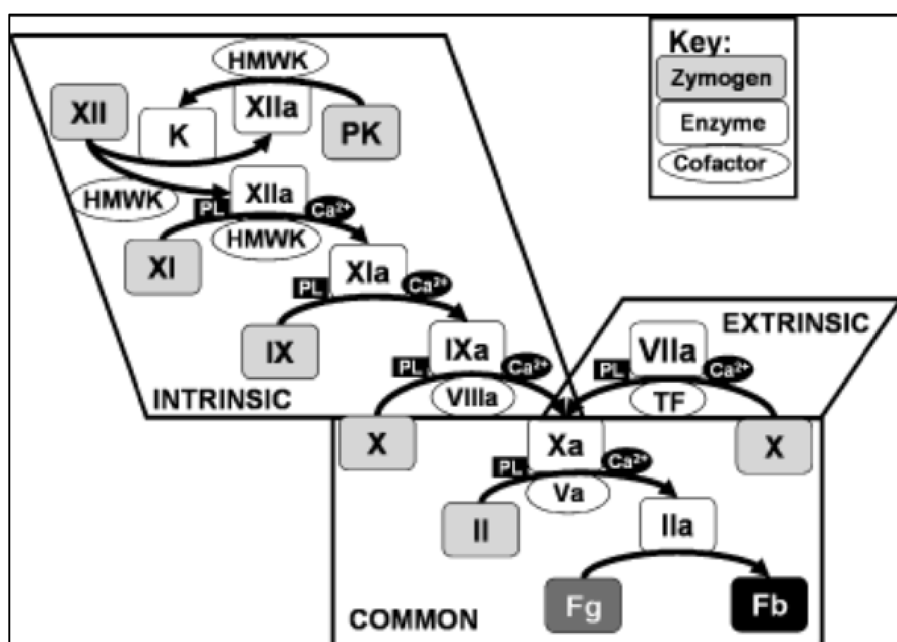

FIGURE 1: THE CASCADE MODEL OF FIBRIN FORMATION[4]

Fb = fibrin, Fg = fibrinogen, HMWK = high molecular weight kininogen, K = kallikrein, PK = prekallikrein, PL = phospholipid, roman numerals refer to coagulation factors.

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This model (Figure 1) is useful to understand plasma-based *in vitro* coagulation. It defines a series of steps where zymogens (coagulation proteins) are cleaved by enzymes (activated coagulation proteins) and co-factors to form the next enzyme in the cascade. These plasma components and steps were thought to represent two independent and redundant pathways: extrinsic and intrinsic. The extrinsic pathway starts with activation of Factor VII in blood by tissue factor (TF) (see later) from the extravascular space.[4-7] The intrinsic pathway (also known as contact activation system) starts with contact activation of Factor XII by a negatively charged surface from the intravascular space, a process that

is approximately 100 times faster when kallikrein is present.[8] These pathways converge with the production of Factor Xa and the start of the common pathway, which ends in the production of fibrin.[4-7]

PT and aPTT are plasma-based assays used to assess dysfunction in the cascade model and will be discussed later.

In vivo haemostasis is more complex than this cascade model can explain. For example, a deficiency in Factor XII (an initial component of the intrinsic pathway) causes a prolonged aPTT but no bleeding tendency in humans.[9] Some mammals, such as whales and dolphins do not even have this protein.[10] Additionally, this model does not take the role of platelets into account, an essential component in primary haemostasis. The cascade model has thus become outdated and replaced by the cell based model of haemostasis.[4]

2.3 The cell-based model of haemostasis

(a) INITIATION
TFPI Xa
AT Xa
V
IIa
TF VIIa X Xa Va II
TF Bearing Cell
TF VIIa IX IXa
(b) AMPLIFICATION
IIa VIII vWF
TF Bearing Cell
Va Xa II
VIIIa vWF
Platelet adhesion
IIa XI
IIa
V V
XIa
Va Platelet
Activated Platelet
(c) PROPAGATION
IIa
XIa IX VIIIa IXa X Va Xa II
Activated Platelet

FIGURE 2: THE CELL-BASED MODEL OF FIBRIN FORMATION[4]

AT = antithrombin, TF = tissue factor, TFPI = tissue factor pathway inhibitor, vWF = von Willebrand factor

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This newer model (Figure 2) consists of three overlapping phases: initiation, amplification and propagation.[11] It incorporates plasma-based coagulation proteins and co-factors with two cell types: TF-bearing cells and platelets.[12]

TF is currently accepted to be the sole initiating factor of *in vivo* haemostasis.[12-14] It is the only coagulation protein that is permanently attached to the surface of a cell membrane.[12,13] Other coagulation proteins circulate and require Ca^{2+} and an active membrane surface to attach to.[4] Endothelial cells contain TF, which is not in contact with blood until injury occurs. TF-bearing cells predominately outside the vasculature include stromal fibroblasts, macrophages and mononuclear cells.[15] The circulation of TF-bearing monocytes, as well as the discovery of TF-bearing platelets, means that haemostasis can occur with an intact endothelium.[16,17] The clinical implication of this is unknown.

2.3.1 Initiation phase

This phase occurs on a TF-bearing cell. Factor VIIa is the only coagulation protein that circulates in plasma in its active enzyme form.[18] When blood and TF-bearing cells make contact following injury, the extrinsic pathway is initiated and Factor VIIa binds to the exposed TF.[13] The TF-FVIIa complex activates more Factor VII to Factor VIIa.[4] Increased TF-FVIIa activity then activates small amounts of Factor IX and Factor X on the TF-bearing cell.[12] Factor Xa slowly directly activates Factor V.[19] FXa-FVa ("prothrombinase") complex activates prothrombin (Factor II) to small amounts of thrombin (Factor IIa). Any Factor Xa that dissociates from the TF-bearing cell will be rapidly inactivated by antithrombin (AT) or tissue factor pathway inhibitor (TFPI). Factor IXa that dissociates can bind to nearby platelets or other cells. It is inactivated more slowly by AT than Factor Xa, and is not inactivated by TFPI.[12]

2.3.2 Amplification phase

The small amount of thrombin produced on the TF-bearing cell during the initiation phase detaches and executes five functions in the amplification phase. Firstly, it activates platelets.[12] Secondly and thirdly, on the platelet surface, it activates Factor V to Factor Va and Factor XI to Factor XIa. Fourthly, it separates vWF from Factor VIII, allowing vWF to mediate additional platelet aggregation. Fifthly, it activates Factor VIII to Factor VIIIa.[12,20] Activated platelets "flip" – shuffling negative membrane phospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE) to the outside, thereby creating a procoagulant membrane which supports the binding of coagulation proteins.[4] Activated platelets release three types of granules: α granules, dense granules and lysosomes. Alpha granules contain mainly proteins which recruit additional platelets and promote platelet activation, as well as play a role in inflammation and wound healing. Dense granules contain polyphosphate (PolyP) which has been postulated to initiate the intrinsic pathway via contact activation of Factor XII to Factor XIIa, however, the contribution of this to haemostasis is debated.[21-24]

2.3.3 Propagation phase

This phase occurs on platelets. Factor IXa produced by the TF-FVIIa complex on the TF-bearing cell in the initiation stage attaches to the activated, "flipped", platelet surfaces. Additional Factor IXa is produced when Factor IX is activated by Factor XIa produced in the amplification stage. Factor VIIIa, also produced in the amplification stage, attaches to Factor IXa on the platelet surface to form a FVIIIa-FIXa ("tenase") complex. Factor X attaches to the platelet surface and is activated by FVIIIa-FIXa to Factor Xa. Factor Xa then attaches to Factor Va produced in the amplification

stage to form a FXa-FVa ("prothrombinase") complex (as is found in the initiation stage) which activates prothrombin to thrombin. Thrombin cleaves fibrinopeptide A ("fibrin") from fibrinogen. The burst of thrombin, and consequent fibrinopeptide A production, reaches a critical point where soluble fibrin molecules spontaneously polymerize into insoluble fibrin strands and consequent gel ("clot").[4,12]

2.3.4 Haemostasis after clot formation

Haemostasis does not end after the formation of a fibrin clot. Approximately 95% of thrombin formation happens after clot intiation.[25] In addition to fibrinopeptide A, thrombin cleaves fibrinopeptide B from fibrinogen. Fibrinopeptide B may not be necessary for fibrin polymerization but aids in the alignment of strands in the fibrin gel.[26] Thrombin also activates Factor XIII to Factor XIIIa which enhances crosslinking of the fibrin gel, increasing stability and resistance to fibrinolysis.[27] The importance of this crosslinking is observed in humans with a Factor XIII deficiency and consequent haemorrhagic phenotype.[28] Some thrombin will bind to thrombomodulin (TM) found on the surface of adjacent intact endothelial cells. The thrombin-TM complex has two functions. Firstly, it activates thrombin activatable fibrinolysis inhibitor (TAFI). This enzyme removes terminal lysine residues (binding site of several fibrinolytic proteins) of fibrin molecules, increasing resistance to fibrinolysis.[29] Secondly, it activates protein C (PC) to form aPC.[12]

2.3.5 Restricting haemostasis to the site of injury

It is vital that haemostasis is restricted to the site of injury, and there are processes in place to ensure this, referred to by some as a "termination phase".[12] Firstly, aPC (as mentioned above) attaches to protein S (ProS) to form the aPC-ProS complex. This complex inactivates Factor Va and Factor VIIIa, thereby shutting down the tenase and prothrombinase complexes produced in the propagation stage and inhibiting new thrombin production. The inactivation of Factor Va is much more efficient on adjacent, healthy endothelial cells than on platelets.[30] Secondly, AT and TFPI are always present on intact endothelial surfaces.[12]

2.3.6 Fibrinolysis

Haemostasis and fibrinolysis are concurrently activated. Fibrinolysis is the enzymatic process that dissolves the fibrin "clot". Fibrin-bound plasminogen from the liver is activated by tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA) to produce plasmin. Plasmin breaks fibrin down into fibrin degradation products (FDPs).[31]

2.4 Routine coagulation assays

Prothrombin time (PT) is a test designed to mimic the extrinsic pathway of the cascade model of coagulation. Thromboplastin (source of TF) and calcium are added to citrated plasma. Time to clot formation is measured.

Activated partial thromboplastin time (aPTT) is a test designed to mimic the intrinsic pathway of the cascade model of coagulation. Kaolin (contact-dependent activator of Factor XII), cephalin (negatively charged surface) and calcium are added to citrated plasma. Time to clot formation is measured.

For both PT and aPTT, observation of fibrin gel is the end point, rendering them unable to detect haemostatic deficiencies past this point.[25] Being plasma-based tests, they do not take platelets or TF-bearing cells into account, two integral parts in the amplification and propagation phases of coagulation, as described in the cell-based model above.

2.5 Thromboelastography

2.5.1 Introduction to thromboelastography

Thromboelastography (TEG) is an analytical method for assessment of haemostasis that was first introduced in the 1940s by Hartett.[32] TEG analyses haemostasis more extensively (includes cellular and soluble components) than routine coagulation assays, providing a continuous detection and recording of viscoelastic changes in whole blood during clot initiation, amplification, propagation and lysis (Figure 1).[11,33] The methodology involved in TEG is as follows: Whole blood (with/without activation) is added to a pre-heated (37°C) cup that contains $CaCl_2$. A pin attached to a torsion wire is introduced into the cup after which the cup initiates movement. As the blood begins to clot, tension exerted on the torsion wire translates to an electrical signal that produces a tracing (Figure 3). The tracing consists of an x-axis (time in seconds) and y-axis (amplitude of torsion wire oscillation in millimeters).[34] The components of this tracing are explained further in the Thromboelastography Variables section below.

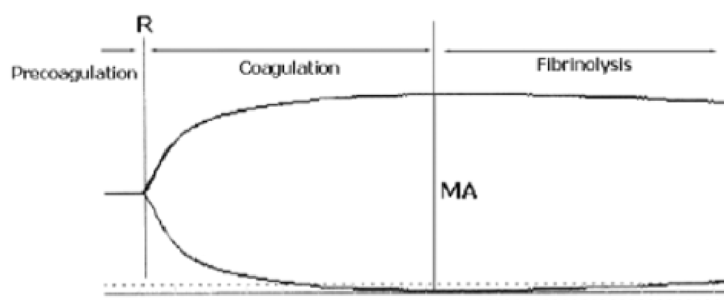

FIGURE 3: DIAGRAM OF A TYPICAL TEG TRACING SHOWING CLOT INITIATION, AMPLIFICATION, PROPAGATION AND LYSIS. [3]

(Reprinted from Journal of Veterinary Emergency and Critical Care 15(1), SM Donahue, CM Otto, Thromboelastography: a tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis © Veterinary Emergency and Critical Care Society 2005, with permission from John Wiley and Sons, Inc.)

TEG was originally designed to be a bed-side, point-of-care analysis of non-anticoagulated ("fresh") whole blood. Fresh whole blood had to be placed in the analyser within 4 minutes after venipuncture (before significant coagulation starts in the specimen), which made it impossible to perform the test when the analyser was not in close vicinity to the patient.[35]

As previously mentioned, Ca^{2+} is essential to the activity of coagulation proteins. When whole blood is collected in a sodium citrate tube, available Ca^{2+} binds to citrate, which in theory inhibits coagulation and thrombin formation. It has, however, been shown that this inhibition is incomplete and some thrombin is actually formed during citrate storage, thereby affecting TEG readings.[36] TEG using fresh whole blood may yield a more accurate *in vivo* representation of coagulation than citrated blood, however, the use of recalcified citrated blood with/without activation has increased the time from venipuncture to analysis to a more practical 30 minutes.[35] It is important to note that TEG results generated using fresh versus citrated whole blood are non-comparable. Laboratory protocols and interpretations thus require standardization based on the sampling conditions.[37]

2.5.2 Thromboelastography variables

There are four standard variables generated from a thromboelastograph (Figure 4):[33,34]

- Reaction time (R): This represents time between clot initiation and the production of the initial insoluble fibrin strands ("clot") and is proportional to coagulation protein level.[38]
- Clot formation time (K): This represents the time between initial clot formation and a pre-determined strength of clot formation.
- Clot angle (α): This represents the acceleration of fibrin formation and cross-linking.
- Maximum amplitude (MA): This represents maximum clot strength and is proportional to platelet count, platelet function and fibrinogen level.[39-42]

Other values, including global clot strength (G; a modification of MA) and clot lysis (LY30 and LY60; representing the percentage of clot lysis detected 30/60 minutes after MA was reached), can also be generated.[34]

FIGURE 4: THROMBOELASTOGRAPH VARIABLES[3]

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2.5.3 Thromboelastography without activation

The initiation of coagulation in inactivated ("native") blood samples is like that of kaolin/celite in the sense that there is negatively charged contact activation of Factor XII to Factor XIIa, however, it is the TEG cup that provides this contact.[43]

2.5.4 Thromboelastography with activation

The use of activation both reduces the time it takes for TEG tracings to be generated, making it more practical in an intra-operative or emergency setting[44], as well as reduces sensitivity to pre-analytical limitations (mentioned below).[45] Activators accepted and described in the literature include recombinant tissue factor (rTF), kaolin and celite.[46-48]

rTF activation mimics the role of endogenous TF, the primary stimulus of initiating *in vivo* coagulation, by activation of Factor VII and Factor X.[49]

Kaolin and celite are both anionic. The addition of either in a TEG assay activates Factor XII to Factor XIIa through contact activation (intrinsic pathway) with a negatively charged surface. Factor XIIa activates Factor XI, which activates Factor IX, which activates Factor X.[19]

2.5.5 Use of thromboelastography in human medicine

In human medicine, TEG is widely used for detecting hypercoagulable or hypocoagulable states, monitoring anticoagulant therapy and evaluating the effect of transfusions during cardiac or hepatic surgery.[50,51]

2.5.6 Use of thromboelastography in veterinary medicine

In veterinary medicine, TEG has been validated for use in dogs, cats, horses and cattle, as well as several non-domestic species.[52-59] Examples of completed research to date into TEG and its diagnostic and therapeutic utility in conditions and treatment regimens include: canine hyperadrenocorticism; endotoxaemia in dogs, canine spirocercosis, uncomplicated canine babesiosis (*Babesia rossi*), protein-losing nephropathy in dogs, acute liver disease in dogs, chronic hepatopathies in dogs, measuring anticoagulant effects of rivaroxaban in dogs, neoplasia in dogs, protein-losing enteropathy in dogs, *Ehrlichia canis* infection in dogs, congenital portosystemic shunts in dogs, ischemic or inflammatory gastrointestinal disease in horses, disseminated intravascular coagulation in dogs and sick non-septic and septic neonatal foals.[60-77]

2.5.7 Pre-analytical limitations of thromboelastography

In South Africa, the only facility that offers TEG assays for veterinary patients (mainly dogs and horses) is the Clinical Pathology Laboratory located at the Faculty of Veterinary Science, University of Pretoria. The current laboratory standard operating procedure (SOP) utilizes kaolin-activated, recalcified citrated whole blood stored at room temperature (defined as 22°C) which is analysed 30 minutes after collection. This SOP was originally formulated for TF-activated TEG, based on recommendations from a 2014 systematic review[1], and storage time was kept at 30 minutes when the laboratory changed over to kaolin-activated TEG in 2016.

The current protocol is subject to two limitations, namely time and temperature. The Onderstepoort Veterinary Academic Hospital (OVAH, located in the same building at the Clinical Pathology Laboratory) is one of the only clinical facilities that can deliver the samples to the TEG analyser on time. There are a few smaller veterinary clinics within a 30-minute radius of the Clinical Pathology Laboratory, but this introduces the second limitation: temperature. South Africa experiences very warm summer months and mild winter months, thus keeping samples at the recommended temperature of 22°C is challenging outside of a hospital setting.

The situation in South Africa is merely an example and these limitations are experienced globally. Increasing the amount of time allowed between blood collection and analysis, as well as extending the range of the allowed storage temperature will make TEG a more accessible tool in the veterinary and medical fields. Storage studies are imperative to achieve this.

2.5.8 Thromboelastography storage studies in human medicine

The following information is summarized in Table 1.

There are multiple TEG storage studies in human medicine. For the sake of relevance to our study, only four studies that utilized kaolin will be reviewed.

Johannson et al. (2008) evaluated kaolin-activated TEG sample stability in 7 healthy blood donors. All samples were stored at room temperature (not specified). The first TEG analysis was performed at 0 minutes, followed by analysis at 30, 60 and 90 minutes. K, G, LY30 and LY60 value were not recorded or evaluated. Statistically, R significantly decreased at 30 and 90 minutes and α significantly increased at 60 and 90 minutes, compared to 0 minutes. Clinical relevance using reference intervals was not assessed.[78]

White et al. (2010) evaluated kaolin-activated TEG sample stability in 61 intensive care (unhealthy) patients. Arterial blood was collected. All samples were stored at room temperature. The first TEG analysis was performed at 15 minutes, followed by analysis at 30 minutes and two hours. G and LY60 value were not recorded or evaluated. Statistically, R and MA decreased at two hours compared to 30 minutes. Clinical relevance using reference intervals was not assessed.[79]

Tuovila et al. (2017) evaluated kaolin-activated TEG sample stability in 51 patients. All samples were stored at room temperature (not specified). Only R and MA values were recorded and evaluated. The first TEG analysis was performed at 30 minutes, followed by analysis at 60 minutes and two hours. Comparisons using mean bias showed more differences (increase) in R than MA (increase) at 120 minutes compared to 30 minutes. Clinical relevance using references intervals was not assessed.[80]

Dias et al. (2017) evaluated kaolin-activated TEG sample stability in 20 healthy blood donors. All samples were stored lying horizontally at room temperature (not specified). The first TEG analysis was performed at 15 minutes, followed by analysis at 30, 60 and 120 minutes. G and LY60 value were not recorded or evaluated. Statistically, K significantly decreased and α significantly increased at 120 minutes compared to 15 minutes. Clinically, all results were within the reference intervals.[81]

TABLE 1: SUMMARY OF CITRATED WHOLE BLOOD KAOLIN-ACTIVATED THROMBOELASTOGRAPHY STORAGE STUDIES IN HUMAN MEDICINE

	Nr of study subjects	Storage times	Storage temperature	Variables recorded and evaluated	Statistically significant differences	Clinically relevant differences
Johannson et al. (2008)[78]	7	0 minutes compared to 30, 60 and 90 minutes	Room (not specified)	R, α and MA	30 minutes: Decreased R 60 minutes: Increased α 90 minutes: Decreased R and increased α	Not assessed
White et al. (2010)[79]	61	15 minutes compared to 30 and 120 minutes	Room (not specified)	R, K, α, MA and LY30	Two hours compared to 30 minutes: Decreased R and MA	Not assessed
Tuovila et al. (2017)[80]	51	30 minutes compared to 60 and 120 minutes	Room (not specified)	R and MA	Not assessed. Comparisons using mean bias only.	Not assessed
Dias et al. (2017)[81]	20	15 minutes compared to 30, 60 and 120 minutes	Room (not specified)	R, K, α, MA and LY30	120 minutes: Decreased K and increased α	None

2.5.9 Thromboelastography storage studies in veterinary medicine

The following information is summarised in Table 2.

To our knowledge, there is only one kaolin-activated TEG storage study congress abstract in dogs and one published kaolin-activated TEG storage study in horses. Dairy cows and Asian elephants also have one published kaolin-activated storage study each.

Sommerey et al. (2013) evaluated kaolin-activated TEG sample stability in 16 healthy dogs (breed undefined). All samples were stored at room temperature (18°C). The first TEG analysis was performed within 2 hours, followed by analysis between 21 and 69 hours. G, LY30 and LY60 value were not recorded or evaluated. Visual inspection of TEG graphs showed a good similarity between 2 hours and 24 hours, and acceptable similarity between 2 hours and 48 hours. Statistical differences were not mentioned. Clinically, only one dog showed a difference at 47 hours, but the nature of the difference is not mentioned. The authors concluded that samples appear to be stable for kaolin-activated TEG in dogs up to 36 hours post-sampling, although their baseline for the first TEG analysis was 2 hours, not 30 minutes.[82]

Machackova et al. (2018) evaluated kaolin-activated TEG sample stability in seven healthy horses (breed undefined). All samples were stored at room temperature (21°C). The first TEG analysis was performed within 2 hours, followed by analysis at 6, 12 and 24 hours. G, LY30 and LY60 values were not recorded or evaluated. Statistically, R significantly decreased at 12 and 24 hours. Clinically, three R values were below-, two K values were above-, two α values were below- and no MA values were outside the reference intervals. Whilst these results are conflicting in terms of inferring hypo- or hypercoagulability, they exhibit a trend. Again, the baseline for the first TEG analysis was 2 hours, and differences between 30 minutes post-sampling and subsequent time points were not evaluated.[83]

To our knowledge, in animal species other than dogs and horses, there are two published kaolin-activated TEG storage studies.

Sommerey et al. (2014) evaluated kaolin-activated TEG sample stability in 28 healthy dairy cows. All samples were stored at room temperature (not specified) and the first TEG analysis was performed within 2 hours. All 28 samples were analysed again at 16-77 hours and 7 samples were analysed a third time at 16-100 hours. LY30 and LY60 were not evaluated. Statistically, R significantly decreased after two hours. Clinically, only two R values were slightly below the reference interval.[59]

Perrin et al. (2018) evaluated kaolin-activated TEG sample stability in 36 healthy Asian elephants. Samples were stored at room temperature (not specified) and the first TEG analysis was performed 60 minutes post-sampling. Samples were then transferred to the fridge (2-4°C) where they were stored until 23.5 hours post-sampling. Samples were then allowed to return to room temperature before analysis at 24 hours post-sampling. LY30 and LY60 were not evaluated. Statistically, R, K, MA and G significantly decreased and α significantly increased at 24 hours. Clinically, some R values were below-, some α values were above-, some MA values were below-, some G values were below- and no K values were outside the reference intervals.[84]

In terms of the rTF-activation method, there is one published rTF-activated TEG storage study in dogs and one in horses.

Wiinberg et al. (2005) evaluated rTF-activated TEG sample stability in 18 healthy dogs. All samples were stored at room temperature (20°C). The first TEG analysis was performed at 30 minutes, followed by analysis at two hours. G, LY30 and LY60 value were not recorded or evaluated. Statistical differences were assessed via coefficients of variation (CVs) or arithmetic means. There was a tendency towards hypercoagulability at two hours, with a decreased R and K and an increased α and MA. Clinical relevance using reference intervals was not assessed.[58]

Leclere et al. (2009) evaluated rTF-activated TEG sample stability in 13 healthy horses (standardbreds, quarter horses and cross breeds). All samples were stored at room temperature (22-25°C). The first TEG analysis was performed at 30 minutes, followed by analysis at 60 and 120 minutes. G, LY30 and LY60 value were not recorded or evaluated. Statistically, R and K significantly decreased and α significantly increased at/after 60 minutes. Clinical relevance using references intervals was not assessed.[55]

TABLE 2: SUMMARY OF CITRATED WHOLE BLOOD THROMBOELASTOGRAPHY STORAGE STUDIES IN VETERINARY MEDICINE

	Activator	Nr of study subjects and species	Storage times	Storage temperature	Variables recorded and evaluated	Statistically significant differences	Clinically relevant differences
Sommerey et al. (2013)[82]	Kaolin	16 dogs	Two hours compared to 21-69 hours	Room (18°C)	R, K, α and MA	Not assessed	47 hours: one dog. Not specified
Machackova et al. (2018)[83]	Kaolin	Seven horses	Two hours compared to six, 12 and 24 hours	Room (21°C)	R, K, α and MA	12 and 24 hours: decreased R	Three R values and two α values decreased (below RI). Two K values increased (above RI)
Sommerey et al. (2014)[59]	Kaolin	28 cows	Two hours compared to 16-100 hours	Room (not specified)	R, K, α, MA and G	After two hours: decreased R	Two R values decreased (below RI)
Perrin et al. (2018)[84]	Kaolin	36 elephants	60 minutes compared to 24 hours	Fridge (2-4°C)	R, K, α, MA and G	Decreased R, K, MA and G Increased α	Some R, MA and G values decreased (below RI) Some α values increased (above RI)
Wiinberg et al. (2005)[58]	rTF	18 dogs	30 minutes compared to two hours	Room (20°C)	R, K, α and MA	Decreased R and K Increased α and MA	Not assessed
Leclere et al. (2009)[55]	rTF	13 horses	30 minutes compared to 60 and 120 minutes	Room (22-25°C)	R, K, α and MA	At/after 60 minutes: Decreased R and K and increased α	Not assessed

2.5.10 Clinical relevance in thromboelastography storage studies

Many TEG storage studies only evaluated statistical significance and made no mention of clinical relevance.[55,58,78-80] A statistical difference between groups is not necessarily clinically relevant. As TEG results influence diagnoses, treatments and prognoses in practice, the clinical implication of any changes seen in storage studies, and not only the statistical significance, should be evaluated. Two methods are used to evaluate clinical relevance: the change in results compared to either population-based reference intervals, or to subject-based reference intervals using biological variation.

2.5.10.1 Population-based reference intervals

Population-based reference intervals can be determined by equipment manufacturers or independently by laboratories. Many laboratories create their own reference intervals from a population of healthy individuals found in their catchment area. These populations could comprise of a combination of breeds, sexes, ages. The Clinical Pathology Laboratory located at the Faculty of Veterinary Science, University of Pretoria utilized Thoroughbred horses and registered blood donor dogs (of various breeds) to formulate reference intervals for kaolin-activated TEG. Patient TEG values that lie outside the established reference intervals are interpreted to be abnormal. Cow (Sommerey et al. (2014)[59]) and elephant (Perrin et al. (2018)[84]) TEG storage studies created their own reference intervals to determine clinical significance of results. Dias et al. (2017)[81], a human TEG storage study previously mentioned, utilized established manufacturer's reference intervals to determine clinical significance of results.

2.5.10.2 Subject-based reference intervals

Subject-based reference intervals are created utilizing the concept of biological variation.

Interindividual variability (CV_G) + intraindividual variability (CV_I) = biological variability (CV_B).

Analytical variability (CV_A) + biological variability (CV_B) = total variability (CV_T).[85]

Intraindividual variability (CV_I) is the concept that the same individual can have "varying degrees of normal" based on various pre-analytical factors (time of day being one example), and that values of any measurand will fluctuate around a homeostatic set point. Any value falling outside of this homeostatic range is abnormal for that individual. An analytical system also carries a certain amount of imprecision related to the testing system, called analytical variability (CV_A). Any measured value falling outside of the combined inherent variation of the individual and the assay is considered abnormal. The magnitude of this combined variation can be calculated in order to provide a numerical range which, when exceeded, indicates that a measured value is abnormal. This range is called the reference change value (RCV), or two-sided critical difference (CD) and is determined using the following equation:[85,86]

$$RCV(\%) = 1.96 \times \sqrt{2} \times \sqrt{(CV_I^2 + CV_A^2)}$$

Subject-based reference intervals for each TEG variable from an individual sample can be produced by adding and subtracting the RCV value of that variable to and from the 30 min storage value of that variable. Results outside of this calculated range are considered to have significant implications for clinical interpretation.[85,86]

Analytical variability (CV_A) can be calculated from the results of internal quality control runs.

Laursen et al. (2013)[87] calculated CV_I and RCV values for R, K, α and MA of non-activated, rTF-activated and kaolin-activated citrated whole blood samples from eight healthy adult horses. Wiinberg et al. (2007)[88] calculated intraindividual variations (CV_I) and RCV values for R, SP (split point), α and MA of rTF-activated citrated plasma samples from eight healthy adult beagle dogs.

2.6 Summary

Haemostasis is a complex physiological process incorporating primary haemostasis (platelet aggregation), secondary haemostasis (also known as coagulation; formation of fibrin clot) and fibrinolysis. Primary and secondary haemostasis

is best explained to-date using the cell-based model of haemostasis, replacing the outdated cascade model of coagulation. Thromboelastography is a global assay that analyses haemostasis more extensively (includes cellular and soluble components) than routine coagulation assays (PT and aPTT), providing a continuous detection and recording of viscoelastic changes in whole blood from clot initiation through to lysis. Current limitations on TEG include pre-analytical storage time and temperature, with the current recommendation of 30 minutes storage at room temperature prior to analysis. This limits the use of TEG to practitioners in a 30 minute radius around facilities equipped with TEG analysers. Increasing the amount of time allowed between blood collection and analysis, as well as extending the range of the allowed storage temperature, will allow wider access for field practitioners to TEG analysis, both in South Africa and globally. To date, there are no published kaolin-activated TEG storage studies in dogs and horses that compare the 30 minutes room temperature baseline to varied storage conditions. Published TEG storage studies in human and veterinary medicine suggest a (inconsistent) trend towards hypercoagulability over time.

Chapter 3: Materials and methods

3.1 Experimental design

3.1.1 Study design

This was a prospective observational study.

3.1.2 Study population

Ten clinically healthy dogs and ten clinically healthy horses were included in this study.

3.1.2.1 Inclusion criteria for dogs as study subjects

- Donor for the Onderstepoort Animal Blood Bank (OABB).
- Consent form signed by either OABB (authorised agent) or owner (Appendix A).
- Between 1 year and 9 years old.
- Weighing more than 20 kg.
- Docile and easy to handle to assist with atraumatic venipuncture.
- Any breed and either sex.

3.1.2.2. Inclusion criteria for horses as study subjects

- Donor for the OABB or part of the Onderstepoort Teaching Animal Unit (OTAU).
- Consent form signed by OABB or OTAU (authorised agents) (Appendix B).
- Between 2 years and 25 years old.
- Weighing more than 300 kg.
- Docile and easy to handle to assist with atraumatic venipuncture.
- Any breed and either sex.

3.2 Experimental procedure

3.2.1 Sample collection

All subjects were deemed clinically healthy at the start of the study based on a thorough history and physical examination (Appendix C). Subjects with signs of any inflammatory condition or that had been treated with drugs known to interfere with haemostasis within four weeks prior to the study were not sampled.

Horses were gently restrained in a crush with a halter and lead rein, and dogs were gently restrained in lateral recumbency by trained veterinary personnel, as shown in Figure 5. No chemical restraint was used.

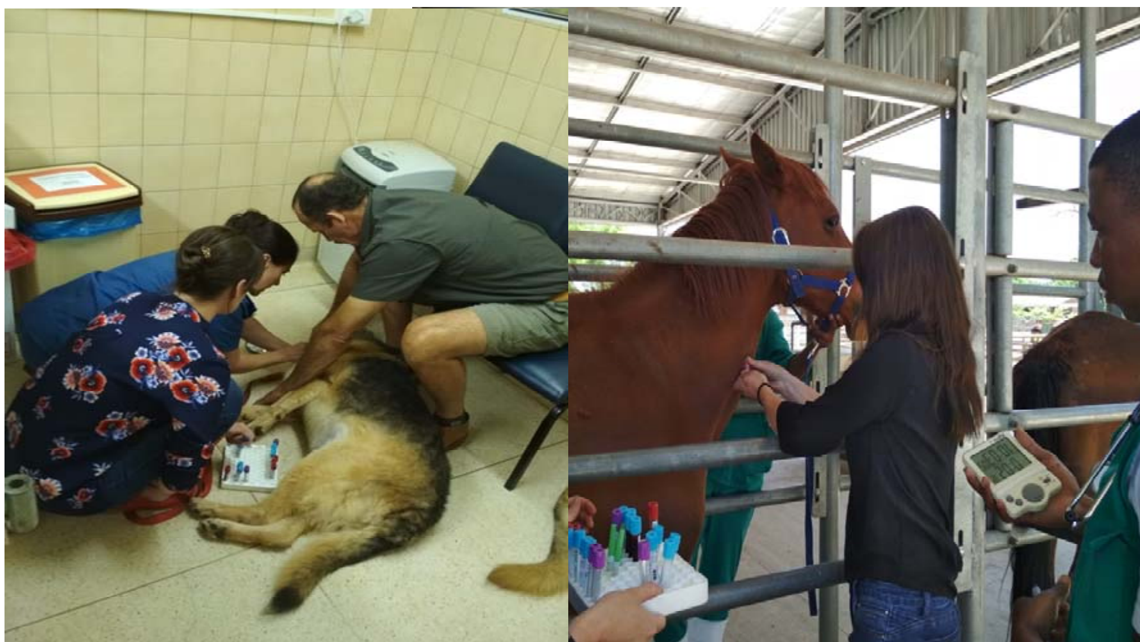

FIGURE 5: GENTLE RESTRAINT AND BLOOD COLLECTION FROM STUDY SUBJECTS (DOGS AND HORSES)

For each subject (dog or horse), blood was collected by the same operator (dogs by MdC and horses by AVL) from the jugular vein, through careful venipuncture, using a 21G needle and vacutainer shoulder. Blood was collected in specific sequence, through vacuum assistance only, into a serum vacutainer tube (discard tube), four 3.2% sodium citrate vacutainer tubes and an EDTA vacutainer tube (BD, Johannesburg, South Africa). The 3.2% sodium citrate tubes were filled to ensure a 1:9 (citrate:blood) dilution, and gently inverted at least three times. The EDTA tube was used to run a complete blood count (CBC) on an automated haematology analyzer (ADVIA 2120i, Siemens Healthcare, Erlangen, Germany) within an hour of sampling. Records were made and kept from each blood collection (Appendix D).

3.2.2 Assay methodologies

3.2.2.1 Haematology analysis

A CBC was performed on the ADVIA 2120i Automated Haematology System (Siemens, Munich, Germany) within one hour of blood collection.

3.2.2.2 Thromboelastography analysis

Of the four citrate tubes, two tubes were stored upright at room temperature (RT) (20-23°C) for up to 22.5 hrs prior to TEG analysis. The remaining two citrate tubes were immediately stored upright in the fridge (FT) (2-7.5°C) for up to 22.5 hrs prior to TEG analysis. For each subject, analyses at the set time points (30 minutes, 2 hrs, 8 hrs and 22.5 hrs) for both room temperature and fridge storage were performed simultaneously. The first two animals (horses) to be sampled had a final storage time of 24 hours. This caused a logistical difficulty during the period of data collection, and the final storage time was consequently changed to 22.5 hrs.

TEG analysis was performed on two TEG 5000 Thrombelastograph Hemostasis System® (Haemonetics Corporation, Braintree, MA, USA) analyzers, as shown in Figure 6, as follows: a sodium citrate tube was gently inverted at least five

times to ensure mixing and then 1 mL of citrated whole blood was added to a kaolin-coated vial. The vial was gently inverted five times after which 340 µL of kaolin-activated blood was added to a pre-warmed sample cup containing 20 µL of 0.2 M $CaCl_2$. The assay was then started. An electronic test and assay of two levels of quality control material were performed daily, and analyses were only performed if the quality control passed the manufacturer's performance goals. Samples stored in the fridge were not allowed to return to room temperature before analysis. R, K, α, MA, LY30 and LY60 variables were recorded.

Figure 6: Two TEG 5000 Thrombelastograph Hemostasis Systems® (Haemonetics Corporation, Braintree, MA, USA) at the Clinical Pathology Laboratory at the Department of companion Animal Clinical Studies at the Faculty of Veterinary Science, University of Pretoria

The TEG analysis was performed by two operators: AVL (horses) and EHH (dogs).

3.3 Observations

All results from the ADVIA 2120 Automated Haematology System (Siemens, Munich, Germany) and TEG 5000 Thrombelastograph Hemostasis System® (Haemonetics Corporation, Braintree, MA, USA) were printed out and stored in the Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria. Data was entered onto an Excel spreadsheet.

3.4 Evaluation of statistical significance

Descriptive statistics were performed, and data was evaluated for normality using the Shapiro-Wilk test. Comparisons between different storage times were evaluated using Friedman's ANOVA with a post-hoc Conover pairwise comparison of variables for non-Gaussian data and a repeated measures ANOVA with a post-hoc Bonferroni correction for Gaussian data. Significance level was set at $p < 0.05$. Statistical analysis was performed using MedCalc Statistical Software version 18.11 (MedCalc Software bvba, Ostend, Belgium).

3.5 Evaluation of clinical relevance

For the dogs, individual results from the different time points were compared to the laboratory's internally generated reference intervals (Table 3). The proportion of results below, within or above the reference intervals were recorded.

TABLE 3: REFERENCE INTERVALS USED BY THE CLINICAL PATHOLOGY LABORATORY AT THE FACULTY OF VETERINARY SCIENCE, UNIVERSITY OF PRETORIA FOR KAOLIN-ACTIVATED THROMBOELASTOGRAPHIC (TEG) ANALYSIS OF DOGS AND HORSES DERIVED FROM CITRATED WHOLE BLOOD SAMPLES STORED FOR 30 MINUTES

	Dogs	Horses
R (min)	2-7	7-17
K (min)	1-4	2-6
α (°)	49-74	34-59
MA (mm)	49-74	48-61
LY30 (%)	0-2	0-1
LY60 (%)	0-8	0-6

For the Nooitgedacht horses, the initial 30 minutes TEG results were often outside of the laboratory's internally generated TEG reference intervals for horses (Table 3). Only Thoroughbred horses were used to generate these reference intervals and a significant breed difference was suspected. Generation of breed-specific reference intervals for Nooitgedacht horses was not within the scope of this study. Therefore, to determine the clinical relevance of any changes for the horses, results were evaluated using subject-based reference intervals by means of the reference change value (RCV).[86] Previously published coefficients of variation for intra-individual variation (CV_I) for R, K, α and MA in horses were used.[87] Analytical imprecision (CV_A) was calculated from the results of the internal quality control runs. The (two-sided) RCV was calculated as:[86]

$$RCV(\%) = 1.96 \times \sqrt{2} \times \sqrt{(CV_I^2 + CV_A^2)}$$

The clinically acceptable deviation away from each 30 minutes TEG variable (R, K, α, MA) was calculated by using the RCV to produce a minimum-maximum range around the 30 minutes result from each horse. Subsequent results from each horse were then evaluated to see whether they fell into this subject-based reference interval. Results outside of the subject's range were considered as having significant implications for clinical interpretation. The clinical relevance of changes in LY30 and LY60 was not assessed as information on biological variation for these variables in horses is not available.

Chapter 4: Results

All study subjects had a normal haematocrit and platelet number, based on automated counting and blood smear evaluation. Estimates of analytical imprecision derived from results of internal quality control measurements were 7.4% for R, 0% for K, 0.8% for α and 5.2% for MA. For some measurements, the sample size was less than ten, due to technical problems with the TEG analyzers.

4.1 Dogs

4.1.1 Statistical significance

The following statistically significant differences were found: shorter R from 30 minutes to 2 hrs RT, 8 hrs RT, 22.5 hrs RT and 22.5 hrs FT; increased K from 30 minutes to 2 hrs FT and 22.5 hrs FT; decreased MA from 30 minutes to 22.5 hrs FT; increased LY60 from 30 minutes to 2 hrs RT, 2 hrs FT and 22.5 hrs FT. α and LY30 values remained stable when stored up to 22.5 hrs RT and FT. No statistically significant differences were found for R, K, α and MA values at 8 hrs FT.

Results for each storage condition (mean or median and range) are shown in Table 4. Graphic comparisons are shown in Figure 7. Statistically significant differences from the 30 minutes room temperature baseline are shown in Table 5.

Table 4: Mean or *median* and (range) for kaolin-activated thromboelastography (TEG) variables in healthy dogs derived from citrated whole blood samples stored under various conditions

	30 min	2 hr RT	2 hr FT	8 hr RT	8 hr FT	22.5 hr RT	22.5 hr FT
R (min)	4.9 (3.1-6.2)	*4.1* (2.3-4.7)*	5.6 (4.5-6.6)	3.1 (1.7-4.3)*	4.2 (3.2-5.7)	3.6 (2.7-4.8)* **	3.2 (2.6-3.9)*
K (min)	*1.7* (1.1-1.8)	1.5 (1.2-2.1)	*1.8* (1.4-2.4)*	1.5 (1.2-1.9)	2.0 (1.3-3.3)	1.6 (1.1-2.8) **	2.3 (1.3-4.3)*
α (°)	66.8 (62.5-73.2)	66.9 (57.4-71.4)	64.4 (58.5-68.3)	69.9 (65.4-73.6)	64.8 (56.7-70.4)	67.7 (55.2-74.6) **	62.8 (53.3-71.5)
MA (mm)	60.1 (54.3-69.7)	60.1 (50.1-65)	*58.1* (56.3-65.8)	60.0 (51.2-68.8)	54.5 (45-65.8)	59.1 (41.4-68.7) **	53.8 (43-62.2)*
LY30 (%)	*0* (0-2.4)	*1.4* (0-17.7)	*0.1* (0-11.5)	*0.05* (0-11.2)	*0* (0-9.2) **	*0* (0-3.2) **	*0.05* (0-27.5)
LY60 (%)	*0.9* (0-7.7) **	*6.1* (1.3-27)*	*2.8* (0-36.3)*	*1.7* (0-29.3)	*2.5* (0-21.5) **	3.7 (0-10.9) ***	*6.9* (0.3-55.6)*

RT room temperature; FT refrigeration temperature

* indicates results significantly different to 30 min values (ANOVA, $p < 0.05$)

Unless otherwise stated, n=10

** n=9

*** n=8

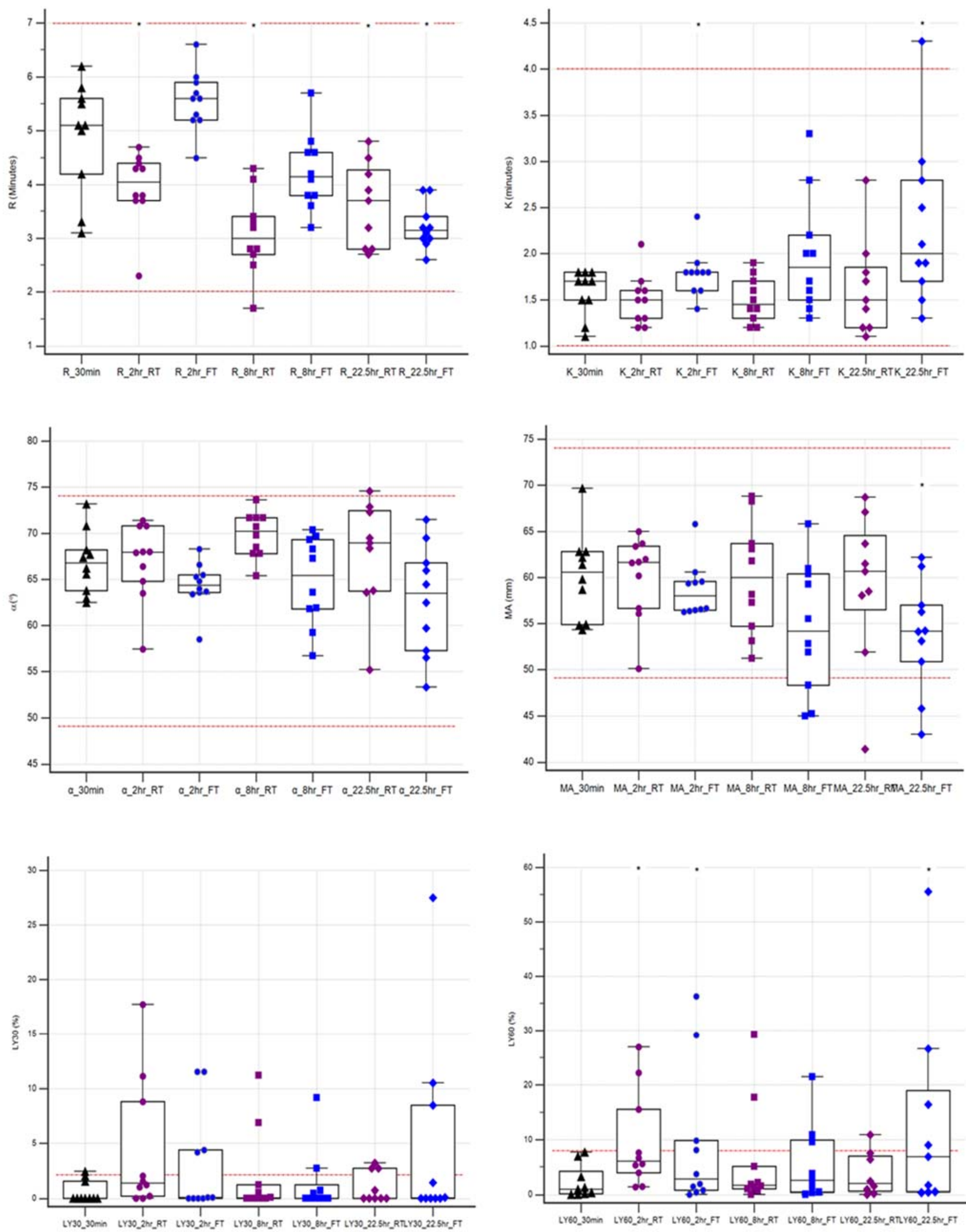

Figure 7: Box-and-whisker plots comparing kaolin-activated thromboelastography (TEG) variables in healthy dogs derived from citrated whole blood samples stored under various conditions. The dotted red lines indicate the reference intervals. * indicates a statistically significant difference from the 30 minutes group. RT room temperature; FT refrigerator temperature

TABLE 5: STATISTICALLY SIGNIFICANT DIFFERENCES FROM THE 30 MINUTES ROOM TEMPERATURE BASELINE IN DOGS

	2 hrs RT	2 hrs FT	8 hrs RT	8 hrs FT	22.5 hrs RT	22.5 hrs FT
R (min)	X		X		X	X
K (min		X				X
α (°)						
MA (mm)						X
LY30 (%)						
LY60 (%)	X	X				X

X = statistically significant difference in at least one subject. RT room temperature; FT refrigeration temperature

4.1.2 Clinical relevance

Barring LY30 and LY60 values, four out of ten samples exhibited clinically relevant differences after 30 minutes when compared to laboratory reference intervals. The proportion of results below, within or above the reference intervals are summarized in Table 6. The first sample exhibited a decreased R (1.7 minutes) at 8 hrs RT and a decreased MA at 8 hrs FT (45.2mm), 22.5 hrs RT (41.4mm) and 22.5 hrs FT (45.8mm). The second sample exhibited a decreased MA (48.3mm) at 8 hrs FT. The third sample exhibited an increased K (4.3 minutes) at 22.5 hrs FT and a decreased MA at 8 hrs FT (45 mm) and 22.5 hrs FT (43 mm). The fourth sample exhibited an increased α (74.6 degrees) at 22.5 hrs RT. R, K, α and MA values for all subjects were within the laboratory reference intervals at 2 hrs RT and 2 hrs FT. For LY30 and LY60, between two and four results were above the upper reference limit at all measurement points after 30 minutes.

Table 6: Number of results below, within or above reference intervals used by the clinical pathology laboratory at the Faculty of Veterinary Science, University of Pretoria for kaolin-activated thromboelastographic analysis in dogs

		n	Below Reference Interval	Within Reference Interval	Above Reference Interval
30 minutes room temperature	R	10		10	
	K	10		10	
	α	10		10	
	MA	10		10	
	LY30	10		9	1
	LY60	9		9	
2 hrs room temperature	R	10		10	
	K	10		10	
	α	10		10	
	MA	10		10	
	LY30	10		7	3
	LY60	10		7	3
2 hrs refrigeration temperature	R	10		10	
	K	10		10	
	α	10		10	
	MA	10		10	
	LY30	10		6	4
	LY60	10		6	4
8 hrs room temperature	R	10	1	9	
	K	10		10	
	α	10		10	
	MA	10		10	
	LY30	10		8	2
	LY60	10		8	2
8 hrs refrigeration temperature	R	10		10	
	K	10		10	
	α	10		10	
	MA	10	3	7	
	LY30	9		7	2
	LY60	9		5	3
22.5 hrs room temperature	R	9		9	
	K	9		9	
	α	9		8	1
	MA	9	1	8	
	LY30	9		6	3
	LY60	8		7	1
22.5 hrs refrigeration temperature	R	10		10	
	K	10		9	1
	α	10		10	
	MA	10	2	8	
	LY30	10		7	3

	LY60	9		5	4

Superimposed TEG tracings were produced for two dogs: the first exhibited no clinically relevant differences in R, K, α and MA after 30 minutes (Figure 8) and the second exhibited clinically relevant differences in R, K, α *or* MA after 30 minutes (Figure 9).

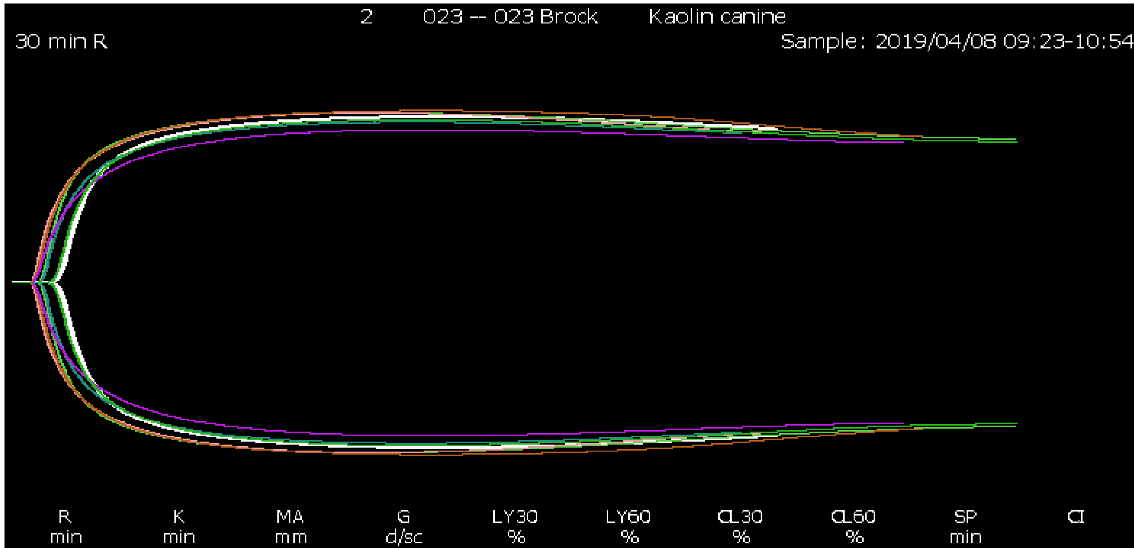

FIGURE 8: SUPERIMPOSED THROMBOELASTOGRAPHY (TEG) TRACINGS OF A DOG (SUBJECT NUMBER 023) PRODUCED AT 30 MINUTES RT (ROOM TEMPERATURE), 2 HOURS RT AND FT (REFRIGERATION TEMPERATURE), 8 HOURS RT AND FT AND 22.5 HOURS RT AND FT. NO CLINICALLY RELEVANT DIFFERENCES IN R, K, ALPHA OR MA AFTER 30 MINUTES WERE NOTED

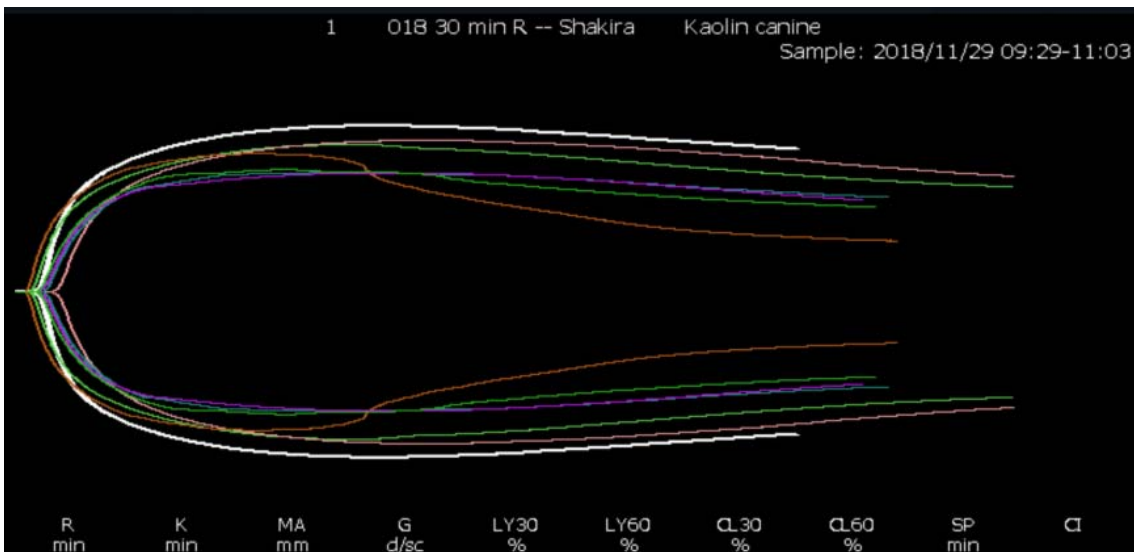

FIGURE 9: SUPERIMPOSED THROMBOELASTOGRAPHY (TEG) TRACINGS OF A DOG (SUBJECT NUMBER 018) PRODUCED AT 30 MINUTES RT (ROOM TEMPERATURE), 2 HOURS RT AND FT (REFRIGERATION TEMPERATURE), 8 HOURS RT AND FT AND 22.5 HOURS RT AND FT. CLINICALLY RELEVANT DIFFERENCES IN R, K, ALPHA AND MA AFTER 30 MINUTES CONSISTED OF A DECREASED R AT 8 HOURS RT AND A DECREASED MA AT 8 HOURS FT, 22.5 HOURS RT AND 22.5 HOURS FT

4.2 Horses

4.2.1 Statistical significance

The following statistically significant differences were found: shorter R from 30 minutes to 2 hrs RT, 8 hrs RT, 22.5 hrs RT and 22.5 hrs FT; decreased K from 30 minutes to 2 hrs RT, 8 hrs RT and 22.5 hrs RT; decreased α from 30 minutes

to 22.5 hrs FT; decreased LY30 from 30 minutes to 8 hrs FT and 22.5 hrs FT; decreased LY60 from 30 minutes to 2 hrs FT, 8 hrs FT, 22.5 hrs RT and 22.5 hrs FT. MA values remained stable when stored up to 22.5 hrs RT and FT. No statistically significant differences were found for R, K, α and MA values at 2 hrs FT and 8 hrs FT.

Results for each storage condition (mean or median and range) are shown in Table 7. Graphic comparisons are shown in Figure 10. Statistically significant differences from the 30 minutes room temperature baseline are shown in Table 8.

TABLE 7: MEAN OR MEDIAN AND (RANGE) FOR KAOLIN-ACTIVATED THROMBOELASTOGRAPHY (TEG) VARIABLES IN HEALTHY HORSES DERIVED FROM CITRATED WHOLE BLOOD SAMPLES STORED UNDER VARIOUS CONDITIONS

	30 min	2 hr RT	2 hr FT	8 hr RT	8 hr FT	22.5 hr RT	22.5 hr FT
R (min)	10 (7.8-11.8)	8.0 (4.9-10.2)*	8.7 (4.5-11.5)	7.3 (4.0-10.0)*	8.1 (5.0-12.3)	7.3 (4.8-10.6)*	5.7 (4.2-7.6)*
K (min)	2.3 (1.8-2.8)	1.9 (1.3-2.8)*	2.5 (1.9-3.6)	1.9 (1.4-2.4)*	2.2 (2.0-3.0)	1.8 (1.3-2.9)*	2.6 (1.8-3.3)
α (°)	60.1 (54.7-67.5)	63.4 (52.8-70.1)	58.9 (47.5-66.1)	61.2 (40.5-71.2)	*60.7* (50.2-62.9)	62.5 (46.6-71.8)	53.8 (43.7-66.4)*
MA (mm)	62.8 (48.4-76.3)	63.3 (45.0-74.2	62.5 (34.0-81.1)	64.2 (44.1-75.3)	63.8 (47.4-72.9)	63.0 (46.6-75.3)	58.5 (48.1-73.2)
LY30 (%)	0.9 (0.0-2.4)	*0.4* (0.0-7.1)	*0.2* (0.0-2.4)	*0.3* (0.0-3.5)	0.0 (0.0)*	*0.2* (0.0-2.9)	*0.0* (0.0-0.3)*
LY60 (%)	4.7 (1.6-7.7)	5.1 (1.7-12.4)	3.6 (1.0-8.5)*	4.3 (1.0-8.5)	*1.4* (0.0-2.0)*	3.7 (0.4-7.6)*	*0.4* (0.0-2.7)*

RT room temperature; FT refrigeration temperature

* indicates results significantly different to 30 min values (ANOVA, $p < 0.05$)

n=10

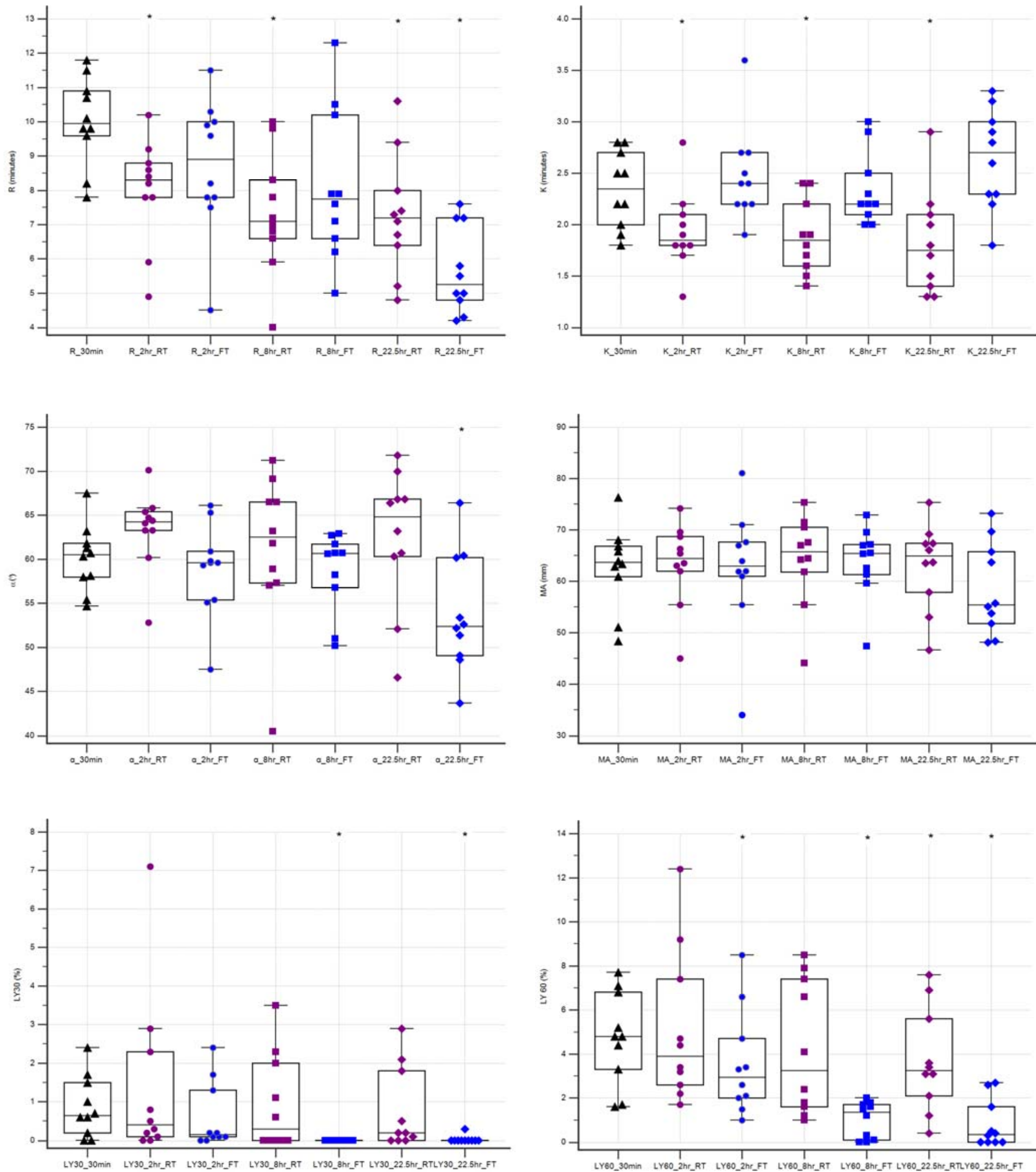

FIGURE 10: BOX-AND-WHISKER PLOTS COMPARING KAOLIN-ACTIVATED THROMBOELASTOGRAPHY (TEG) VARIABLES IN HEALTHY HORSES DERIVED FROM CITRATED WHOLE BLOOD SAMPLES STORED UNDER VARIOUS CONDITIONS. THE DOTTED RED LINES INDICATE THE REFERENCE INTERVALS. * INDICATES A STATISTICALLY SIGNIFICANT DIFFERENCE FROM THE 30 MINUTES GROUP. RT ROOM TEMPERATURE; FT FRIDGE TEMPERATURE

TABLE 8: STATISTICALLY SIGNIFICANT DIFFERENCES FROM THE 30 MINUTES ROOM TEMPERATURE BASELINE IN HORSES

	2 hrs RT	2 hrs FT	8 hrs RT	8 hrs FT	22.5 hrs RT	22.5 hrs FT
R (min)	X		X		X	X
K (min	X		X		X	
α (°)						X
MA (mm)						
LY30 (%)				X		X
LY60 (%)		X		X	X	X

X = statistically significant difference in at least one subject. RT room temperature; FT refrigeration temperature

4.2.2 Clinical relevance

Using subject-based reference intervals based on the RCV[86,87], eight out of ten samples exhibited clinically relevant differences after 30 minutes. The first sample exhibited a decreased MA at 2 hrs FT and a decreased α at 2 hrs FT and 8 hrs FT. The second sample exhibited a decreased α at 8 hrs RT and a decreased MA at 22.5 hrs FT. The third sample exhibited an increased MA at 8 hrs FT. The fourth sample exhibited a decreased α at 22.5 hrs FT, the fifth a decreased α at 22.5 hrs RT and the sixth an increased α at 22.5 hrs RT. The last two of the eight samples exhibited a decreased MA at 22.5 hrs FT. R, K, α and MA values for all subjects were within the subject-based reference intervals at 2 hrs RT. R and K values for all subjects were within the subject-based reference intervals when stored up to 22.5 hrs RT and FT. The clinical significance of changes for LY30 and LY60 were not evaluated as data on biological variation for these variables has not been published.

Superimposed TEG tracings were produced for two horses: the first exhibited no clinically relevant differences in R, K, α and MA after 30 minutes (Figure 11) and the second exhibited clinically relevant differences in R, K, α *or* MA after 30 minutes (Figure 12).

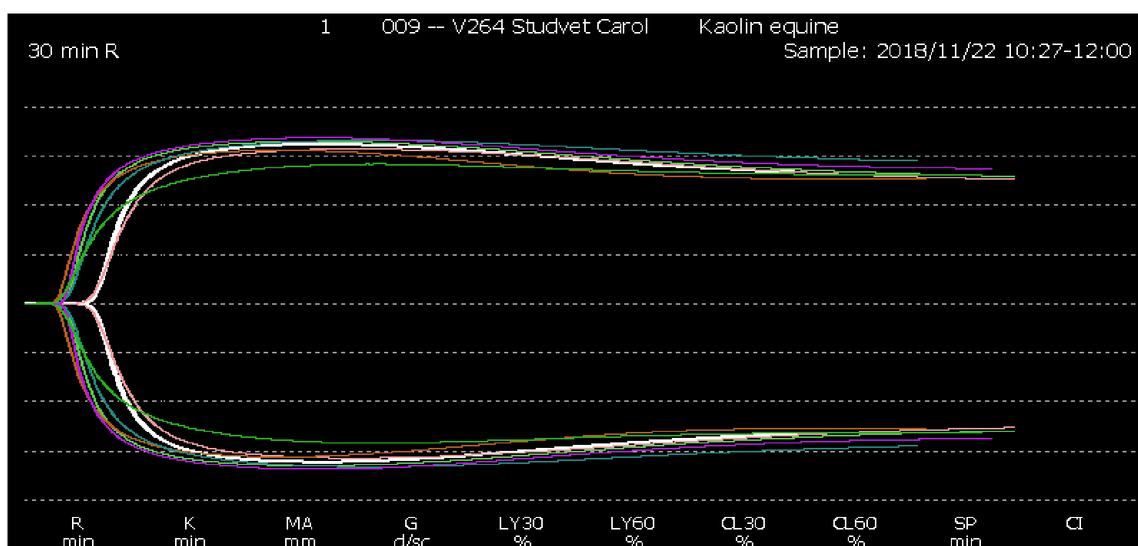

FIGURE 11: SUPERIMPOSED THROMBOELASTOGRAPHY (TEG) TRACINGS OF A HORSE (SUBJECT NUMBER 009) PRODUCED AT 30 MINUTES RT (ROOM TEMPERATURE), 2 HOURS RT AND FT (REFRIGERATION TEMPERATURE), 8 HOURS RT AND FT AND 22.5 HOURS RT AND FT. NO CLINICALLY RELEVANT DIFFERENCES IN R, K, ALPHA OR MA AFTER 30 MINUTES WERE NOTED

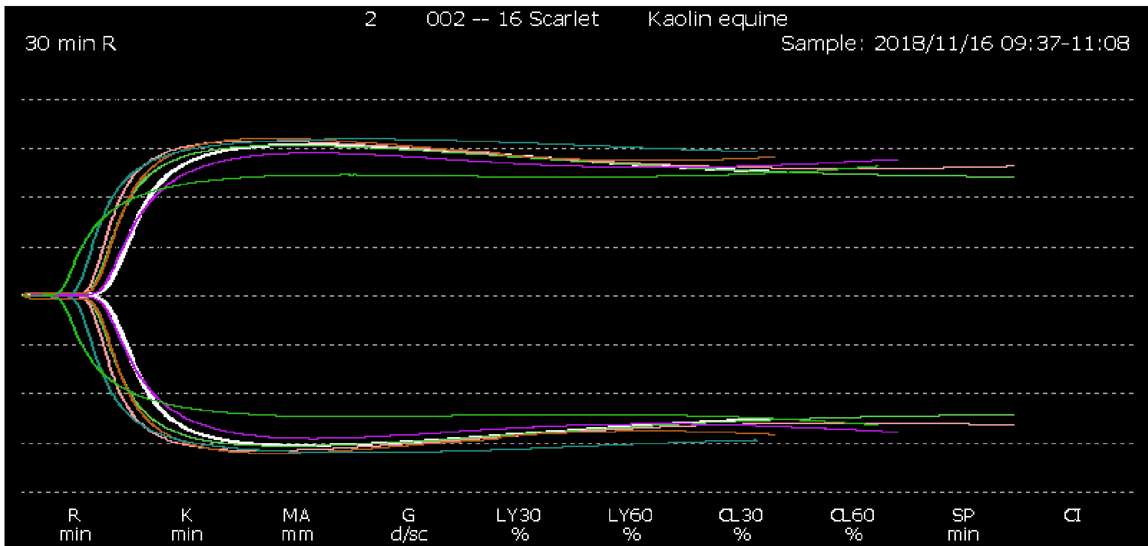

Figure 12: Superimposed Thromboelastography (TEG) tracings of a horse (subject number 002) produced at 30 minutes RT (room temperature), 2 hours RT and FT (Refrigeration temperature), 8 hours RT and FT and 22.5 hours RT and FT. Clinically relevant differences in R, K, alpha and MA after 30 minutes consisted of a decreased alpha at 8 hours RT and a decreased MA at 22.5 hours FT

Chapter 5: Discussion

In our study, for dogs, the four most clinically utilized TEG variables (R, K, α, MA) were stable when samples were stored for up to 8 hrs at FT, apart from K which although stable at 8 hrs FT, showed a small increase at 2 hrs FT. No clinically relevant differences in these variables were noted in samples stored for 2 hrs at RT or FT. Four out of ten samples exhibited clinically relevant differences in these variables after 2 hrs. For horses, R, K, α and MA variables were stable when samples were stored for up to 8 hrs at FT. No clinically relevant differences in these variables were noted in samples stored for 2 hrs at RT. Eight out of ten samples exhibited clinically relevant differences for variables α and MA after 2 hrs RT storage and with refrigeration storage.

In our study, with both dogs and horses, there were often inconsistent findings regarding statistically significant versus clinically relevant differences. For the purpose of this study, where the goal is to facilitate a wider availability of TEG to veterinary general practitioners, consideration of whether storage-induced changes will affect clinical interpretation, is foremost.

As discussed in Chapter 2: Literature review, published storage studies for kaolin-activated TEG in veterinary species (horses, cows and Asian elephants) present conflicting results, probably due to the variation in storage times and species differences. There are no published storage studies for kaolin-activated TEG in dogs. Machackova et al. (2018) evaluated kaolin-activated TEG sample stability at RT in seven healthy horses (breed undefined) and found no statistically significant changes in R, K, α or MA at 6 hours, compared to a 2 hour baseline. Statistically significant decreases in R time were found at 12 and 24 hrs, which is like the trend seen in our study for both dogs and horses. The significant decrease in K from our study was not reported by Machackova et al. Clinically relevant decreases in R, increases in K and decreases α after 6 hrs of storage reported by Machackova et al. were not noted in our study to the same degree.[83] Perrin et al. (2018) recorded significant differences representing a tendency towards hypercoagulability in kaolin-activated TEG samples from 44 Asian elephants after 24 hrs of FT storage (samples were left to come to RT for 30 minutes after 23.5 hours of FT storage), compared to a 60 minute baseline. R, K and MA were significantly decreased and α was significantly increased. Clinically, some R and MA values were below, and some α values were above the reference intervals. In agreement, our study noted a decrease in MA in two dogs and three horses at 22.5 hrs FT. In contrast, our study noted no change in R in dogs or horses, an increased K in one dog subject and a decreased α in one horse subject at 22.5 hrs FT, which may be indicative of elephant-specific variations in haemostasis.[84]

As discussed in Chapter 2: Literature review, published storage studies for kaolin-activated TEG in human medicine also report contradicting findings, reporting either no differences in R or MA [80], or decrease in R, increase in α and no difference in MA [78], or decrease in R and MA and no difference in K, α and LY30 [79], or decrease in K, increase in α and no difference in R, MA and LY30 [78-81]. Those studies did not assess clinically relevant differences. Two storage studies for tissue factor-activated TEG (one in dogs and the other in horses) report corroborative statistically significant

decreases in R and K and increases in α and MA, which corroborates and contradicts trends noted in our study. Those two studies did not assess clinically relevant differences.[55,58]

Apart from a decrease in R, which seems consistent across species and studies, it is not possible to identify an expected storage time-related trend in other variables, which makes interpretation of data and formulation of recommendations difficult. The sample size in most studies, including ours, is small, and outliers representing random error may skew results. High inter-operator variability has been reported for kaolin activated TEG in veterinary medicine, even though the procedure is standardized.[89] High CVs have also been reported within and between veterinary laboratories for kaolin-activated TEG run on canine platelet-rich plasma.[90] These sources of variation may be a reason for the differing results found between studies and species.

In our study, in terms of room or refrigerator storage temperature, neither appeared to have a marked advantage over the other. The effects of cold temperatures on platelet structure, effectivity and longevity have been thoroughly explored and subsequently established. Firstly, soon after refrigeration, platelet shape starts changing from discoid to spherical, indicating activation.[91-93] Secondly, platelet metabolic activity drops due to a decrease in anaerobic glycolysis.[94] Thirdly, aggregation and clot strength have been shown to be enhanced both *in vitro* and *in vivo* in multiple studies [95-99], although, this conclusion has been contradicted by another *in vivo* study that reported equal effectivity of cold and room temperature on platelets for up to 24 hrs [99]. Whilst 24 hour cold temperature storage appears to enhance/maintain platelet function according to the literature, in our study this is not reflected in TEG variables (all except R) which are reported to correlate with platelet count and function [100], where an increased MA, decreased K and increased α would be expected. Studies comparing the effects of cold and room temperature storage on kaolin-activated TEG variables in human blood reported no clinically relevant differences in R, K, α or MA when stored for 24 hrs.[94,101] Direct comparisons between RT and FT at each time point were not made in our study, however, in dogs, two clinically relevant effects of cold temperature storage were noted. Firstly, MA decreased below the lower reference limit in two FT samples at 8 hrs and 22.5 hrs, and in one FT sample at 22.5 hrs only. Secondly, K increased above the upper reference limit in one FT sample at 22.5 hrs. R and α were not affected by cold temperature storage in dogs. In horses, three clinically relevant effects of cold temperature storage were noted. Firstly, MA decreased below the lower reference limit in one FT sample at 2 hrs and three FT samples at 22.5 hrs. Secondly, in stark contradiction to the first, MA increased above the upper reference limit in one FT sample at 8 hrs. Thirdly, α decreased below the lower reference limit in one FT sample at 2 hrs and 8 hrs, and in one FT sample at 22.5 hrs. R and K were not affected by cold temperature storage in horses. A decrease in MA therefore appears to be the most consistent effect of FT storage in our study, which is contradictory to what would be expected if canine and equine platelets are also activated by cold temperatures, as for humans. The reason for this, and for these species' differences in K and α stability is not clear but could be investigated further with fibrinogen and platelet function assays.

As previously mentioned, R represents time between clot initiation and production of initial insoluble fibrin strands ("clot") and is proportional to coagulation protein concentration.[38] In our study, a clinically relevant decrease in R was

seen at 8 hrs RT storage in only one dog and none of the horses. Coagulation time tests, prothrombin time (PT) and activated partial thromboplastin time (aPTT), have been reported to positively correlate with R values.[102] PT and aPTT have also been reported to be respectively prolonged and shortened over 24 hours of canine citrated whole blood storage at RT, but remain unchanged in blood stored at FT [103,104], which is consistent with our findings.

In our study, LY30 and LY60 values were increased above the upper reference limit in several dogs across all storage conditions. Clinically relevant differences in these values were not assessed in horses. Although sufficient evidence for the clinical utility of these lysis variables in dogs is lacking [105], they have been used in dogs to identify hyperfibrinolysis (with or without concurrent hyper- or hypocoagulability) which may accompany trauma [106-108], liver disease [68,109], systemic inflammatory response syndrome (SIRS) [110] or disseminated intravascular coagulopathy (DIC) [75]; or used to monitor fibrinolytic treatment. For previous storage studies, LY30 was either not mentioned [58,59,78,80] or LY30 values were not compared [55,83,84] or LY30 values were compared but showed no statistically significant difference [79,81]. Based on the results of our study, should a clinician specifically want LY30 and LY60 values for dogs, citrated samples should not be stored for longer than 30 min at RT before kaolin-activated TEG analysis.

Limitations of this study include the small sample size, the lack of some TEG data for some subjects and that samples were not analyzed in duplicate, all of which diminish the power of the statistical analysis. In addition, although animals were apparently healthy, full diagnostic and hemostatic testing was not performed to confirm this. In terms of the horses, population-based reference intervals for Nooitgedacht horses were lacking and the CVi used here from a previous publication may not be applicable to our Nooitgedacht population. The longest storage time for samples from two horses was 24 hrs, versus 22.5 hrs for the other eight horses. The results presented here apply to TEG performed on healthy subjects and cannot necessarily be extrapolated to diseased animals, particularly those with hemostatic derangements. Finally, this study did not assess the effect of vibration and extreme temperature variation when transporting samples from the field to the laboratory.

Chapter 6: Conclusions

In healthy dogs, our findings suggest that R, K, α and MA variables can be assessed using kaolin-activated TEG in citrated whole blood samples stored for up to 2 hours at room and refrigerator temperature.

In healthy horses, our findings suggest that R, K, α and MA variables can be assessed using kaolin-activated TEG in citrated whole blood samples stored for up to 2 hours at room temperature. Breed-specific TEG reference intervals need to be created for Nooitgedacht horses (and possibly other horse breeds as well). A follow-up storage study that utilizes breed specific reference intervals should be performed.

The purpose of this study was to define the effects of storage time and temperature on TEG in healthy dogs and horses. Follow-up storage studies should be performed using larger populations of healthy dogs and horses, as well as animals suffering from haemostatic defects. Many of the changes seen in this study cannot easily be explained and contradict results from other storage studies. Incorporating other haemostasis assays like functional fibrinogen concentration and platelet aggregometry could assist in the interpretation of findings in future studies.

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Appendix documents

Appendix A: Consent form for canine study subjects

UNIVERSITEIT VAN PRETORIA
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DEPARTMENT OF COMPANION ANIMAL CLINICAL STUDIES

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

Project title: The effects of storage time and temperature on thromboelastographic analysis in dogs and horses.

(To be completed by the subject's owner / authorised agent)

Please encircle Yes or No where necessary

1. In your opinion, is your dog clinically healthy? Yes / No

2. Do you have ANY concerns regarding the health of your dog? Yes / No

3. Has your dog been on any medication in the last 4 weeks? Yes / No

If you answered yes to the above question, please elaborate ____

4. Do you grant consent that blood samples may be drawn from your dog? Yes / No

5. Have you received detailed information regarding the study? Yes / No

6. Have all the risks involved in the blood sampling procedure (haematoma) been explained to you and do you fully understand them? Yes / No

I hereby give permission that my dog

____

____

__________ (subject's name and unmistakeable distinguishing description) may participate in this study conducted at the Onderstepoort Veterinary Academic Hospital.

I understand that no compensation will be payable to me.

I understand that I will not be liable for any costs of the study.

I agree that this form serves to fully indemnify the University of Pretoria and the researcher(s) against any future claims resulting from the blood collecting procedure.

I understand that no personal information will be disclosed but may be used anonymously in publications.

I understand that it is my right to withdraw my animal from the study.

Signed at Onderstepoort on the _________________ day of _________________ 20______

Signature Owner/Authorised Agent ____

Home Tel: _____________________________________ Work Tel: _____________________________________

Cell No: ____

Signature Dr Ashleigh Lemon (Principle Investigator): ____

Signature Witness: ____

We thoroughly appreciate your willingness to participate in this study. If you have any questions, please feel free to contact me (Dr Ashleigh Lemon) at ashleighlemon@gmail.com, or the Clinical Pathology Department at OVAH.

Appendix B: Consent form for equine study subjects

DEPARTMENT OF COMPANION ANIMAL CLINICAL STUDIES

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

Project title: The effects of storage time and temperature on thromboelastographic analysis in dogs and horses.

(To be completed by the subject's owner / authorised agent)

Please encircle Yes or No where necessary

1. In your opinion, is your horse clinically healthy? Yes / No

2. Do you have ANY concerns regarding the health of your horse? Yes / No

3. Has your horse been on any medication in the last 4 weeks? Yes / No

If you answered yes to the above question, please elaborate ____

4. Do you grant consent that blood samples may be drawn from your horse? Yes / No

5. Have you received detailed information regarding the study? Yes / No

6. Have all the risks involved in the blood sampling procedure (haematoma) been explained to you and do you fully understand them? Yes / No

I hereby give permission that my horse

____

____

__________ (subject's name and unmistakeable distinguishing description) may participate in this study conducted at the Onderstepoort Veterinary Academic Hospital.

I understand that no compensation will be payable to me.

I understand that I will not be liable for any costs of the study.

I agree that this form serves to fully indemnify the University of Pretoria and the researcher(s) against any future claims resulting from the blood collecting procedure.

I understand that no personal information will be disclosed but may be used anonymously in publications.

I understand that it is my right to withdraw my animal from the study.

Signed at Onderstepoort on the ________________ day of ________________ 20_____

Signature Owner/Authorised Agent ____

Home Tel: ___________________________________ Work Tel: ___________________________________

Cell No: ______________________________________

Signature Dr Ashleigh Lemon (Principle Investigator): ____

Signature Witness: ____

We thoroughly appreciate your willingness to participate in this study. If you have any questions, please feel free to contact me (Dr Ashleigh Lemon) at ashleighlemon@gmail.com, or the Clinical Pathology Department at OVAH.

Appendix C: Subject information/evaluation sheet

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DEPARTMENT OF COMPANION ANIMAL CLINICAL STUDIES

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

Project title: The effects of storage time and temperature on thromboelastographic analysis in dogs and horses.

Date:

Subject Details:

Subject Number:	
Species:	
Breed:	
Sex:	
Age:	
Weight:	
Name:	

Subject Physical Examination:

Habitus:	
Body Condition Score:	
Temperature:	
Pulse:	
Respiration:	
Mucus Membrane Colour:	
Capillary Refill Time:	
Peripheral Lymph Nodes:	
Abdominal Palpation:	

*Physical Examination performed just before blood collection

Appendix D: Blood collection sheet

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DEPARTMENT OF COMPANION ANIMAL CLINICAL STUDIES

FACULTY OF VETERINARY SCIENCE

UNIVERSITY OF PRETORIA

Project title: The effects of storage time and temperature on thromboelastographic analysis in dogs and horses.

Date:

Subject Number:

Blood Collection: Please circle the correct answer

1. Was the subject docile? Yes / No

2. Was there a single venipuncture? Yes / No

3. Were all blood tubes filled with vacuum assistance alone? Yes / No

4. Were all blood tubes sufficiently filled? Yes / No

5. Were all filled blood tubes inverted 3 times immediately? Yes / No

6. Were all blood tubes filled in the correct order (serum>sodium citrate>EDTA)? Yes / No

7. Did the venipuncture site clot in a normal amount of time? Yes / No

Check List: Please tick

1 Serum Tube ☐

3 Sodium Citrate Tubes ☐

1 EDTA Tube ☐

Appendix E: Invitation to present at ESVCP (European Society of Veterinary Clinical Pathology) Congress in Arnhem, Netherlands 2019

Maastricht, 2 July 2019

Letter of Invitation: 1st joint congress of Veterinary Pathology and Veterinary Clinical Pathology, 25-28 September 2019, Burgers' Zoo, Arnhem, the Netherlands.

Dear Dr. Ashleigh Lemon,

On behalf of the Organizing Committee of the 1st joint congress of Veterinary Pathology and Veterinary Clinical Pathology, we cordially invite you to attend the congress. The 1st joint congress of Veterinary Pathology and Veterinary Clinical Pathology will be held from 25 – 28 September 2019 in Royal Burgers Zoo, Arnhem, the Netherlands.

You are kindly invited for a poster presentations entitled: 'THE EFFECTS OF STORAGE TIME AND TEMPERATURE ON THROMBOELASTOGRAPHIC ANALYSIS IN DOGS AND HORSES.'

Furthermore, we will offer a balanced scientific programme with interesting and attractive topics and speakers, workshops, abstract sessions and clinical case presentations.

Please feel free to use this letter for Visa purposes. The Organizing Committee of the 1th joint congress of Veterinary Pathology and Veterinary Clinical Pathology encourages a broad variety of nationalities. However, this invitation does <u>not</u> constitute any financial obligation on behalf of the organizers for the registration fee, accommodation, travel cost or other budgetary matters.

We hope to have informed you accordingly. If you have any questions, please do not hesitate to contact the Congress Secretariat by e-mail: j.slangen@pauwelspco.nl

Yours sincerely on behalf of the organising committee,

pco

Joeri Slangen
Project Manager

Pauwels Congress Organisers
Avenue Céramique 222
6221 KX Maastricht, the Netherlands

T: +31 (0)43 321 81 80
E: j.slangen@pauwelspco.nl

Appendix F: Poster presented at Faculty Day (22 August 2019) at the Faculty of Veterinary Science, University of Pretoria, and at ESVCP (European Society of Veterinary Clinical Pathology) Congress in Arnhem, Netherlands 2019

The effects of storage time and temperature on thromboelastographic analysis in dogs and horses

A.V. Lemon, A. Goddard, E.H. Hooijberg

Department of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, Pretoria, 0110

Background:

- Thromboelastography (TEG) provides a more extensive analysis of haemostasis than routine coagulation assays.
- The current recommendation in veterinary species is that samples should be stored at room temperature for a maximum of 30 minutes between blood collection and TEG analysis.
- This limitation on storage time and temperature means that general practitioners without immediate access to referral centres cannot use TEG.

Objectives:

To determine whether TEG results are affected by time elapsed between collection and analysis, as well as by storage temperature of samples during this time.

Methods:

- Blood samples taken from 10 healthy dogs (varied breeds and sexes) and 10 healthy horses (Nooitgedacht mares).
- TEG performed on a TEG 5000 Thrombelastograph Hemostasis System® using citrated blood with kaolin activation.
- The first TEG analysis of each subject was performed after 30 minutes of room temperature storage. R, K, α and MA variables were recorded. Values generated were compared to those of samples stored both at room temperature (20-23°C) (RT) and refrigerated (2-7.5°C) (FT) for 2 hours, 8 hours and 22.5 hours.
- Data distribution was evaluated using a Shapiro-Wilk test and group data compared using a Friedman's ANOVA with a post-hoc Conover pairwise comparison of variables (non-parametric data) or a repeated measures ANOVA with a post-hoc Bonferroni correction (parametric data). A P-value of <0.05 was used.
- The clinical relevance of changes was evaluated by comparing results to population-based reference intervals (dogs) or to subject-based reference intervals (reference change value based on biological information data) (horses).

Results:

Dogs:

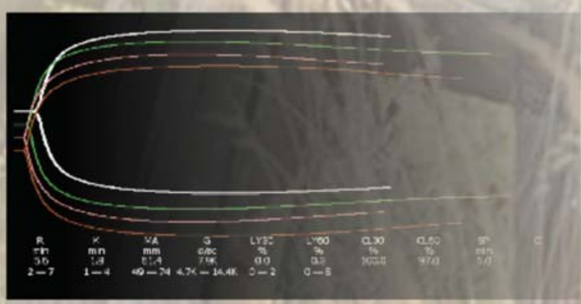
- Results are shown in Figure 1.
- No statistically significant differences were found for R, K, α and MA values at 8 hr FT.
- Clinically relevant differences were noted after 30 minutes in 4/10 subjects, as illustrated by points outside of the reference intervals shown in Figure 1.
- R, K, α and MA values for all subjects were within the laboratory reference intervals at 2 hr RT and 2 hr FT.

Horses:

- Results are shown in Figure 2.
- No statistically significant differences were found for R, K, α and MA values at 2 hr FT and 8 hr FT.
- Clinically relevant differences were noted after 30 minutes in 8/10 subjects when compared to subject-based reference intervals. These included both increased and decreased α and MA.
- R, K, α and MA values for all subjects were within the subject-based reference intervals at 2 hr RT.

Conclusions:

This study suggests that R, K, α and MA variables can be assessed using kaolin-activated TEG in citrated whole blood samples stored for up to two hours at RT or FT in healthy dogs and for up to two hours at RT in healthy horses.



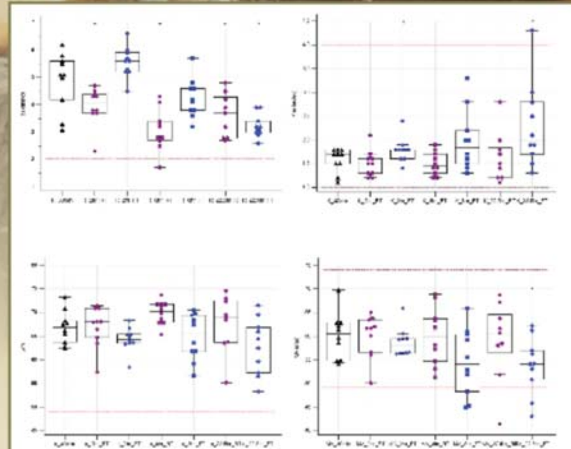


Figure 1: Box-and-whisker plots comparing kaolin-activated thromboelastography (TEG) variables in healthy dogs derived from citrated whole blood samples stored under various conditions. The dotted red lines indicate the reference intervals; * indicates a significant difference from the 30 min group. RT room temperature; FT refrigerator temperature

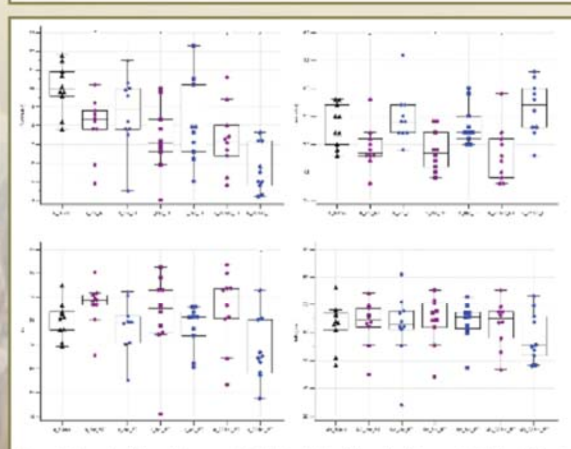


Figure 2: Box-and-whisker plots comparing kaolin-activated thromboelastography (TEG) variables in healthy horses derived from citrated whole blood samples stored under various conditions. * indicates a significant difference from the 30 min group. RT room temperature; FT refrigerator temperature

Appendix G: Awarded "Best Scientific Poster 2019" at Faculty Day (22 August 2019) at the Faculty of Veterinary Science, University of Pretoria