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# Thromboelastography in healthy dairy cows

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## ABSTRACT

Thromboelastography is a whole blood-based coagulation assay that can be used to investigate hypocoagulability and hypercoagulability, as seen with thromboembolic diseases and disseminated intravascular coagulation. Numerous coagulopathies due to different causes are reported in cows. The objective was to establish reference intervals for thromboelastography using the TEG 5000 (Haemonetics GmbH, Munich, Germany) with citrated whole blood samples and kaolin activation in dairy cows and to investigate possible thromboelastographic changes between cows in different lactation periods. An additional objective was to test the stability of samples for up to 100 h. Sixty blood samples from healthy Holstein-Friesian cows were examined. The samples were allocated to 3 different lactation groups (≤30 d postcalving, 31–99 d postcalving, ≥100 d postcalving). Thromboelastography was performed by using the TEG 5000 analyzer with citrated whole blood samples with kaolin activation. The calculated reference intervals were as follows: reaction time = 2.2 to 6.2 min, coagulation time = 0.8 to 2.0 min, angle $\alpha$ = 58.2 to 81.8°, maximum amplitude = 64.3 to 89.2 mm, and clot rigidity = 9.2 to 41.2 dyn/cm$^2$. The 3 different lactation groups showed no significant differences in TEG parameters. No significant difference was seen in samples stored for up to 48 h at room temperature, which indicates that delays in processing samples, such as those arising during transit, are not an issue.
**Key words:** bovine, coagulation, lactation, reference interval, thromboelastography

## INTRODUCTION

Hemostasis is the arrest of bleeding from an injured blood vessel; it requires the combined activity of vascular, platelet, and plasma factors, with regulatory mechanisms to counterbalance the tendency of clots to form. For assessment of hemostasis, coagulation profiles are assessed, including platelet count; fibrinogen concentration; the coagulation times prothrombin time (**PT**), activated partial thromboplastin time (**APTT**), and thrombin clotting time (**TCT**); and single coagulation factors, as well as thromboelastography.

Thromboelastography is a whole blood-based assay used in human and veterinary medicine for evaluation of primary hemostasis, including thrombocytopenia and thrombocytopathy, as well as secondary hemostasis, as seen with hemophilia. Additionally, it is applied to detect hypercoagulability to identify prethrombotic changes, especially early diagnosis of disseminated intravascular coagulation (**DIC**; Donahue and Otto, 2005).

Thromboelastography was first developed by Hartert (1948) to evaluate the coagulation of whole blood. The method assesses the speed and strength of blood clot formation in vitro and is thought to better represent in vivo hemostasis than plasma-based assays (Flint et al., 2011). The principle of the technique is monitoring the gradual binding of a pin to the sides of a cup during clot formation and displaying this as a graph (Wiinberg and Kristensen, 2010). Thromboelastography is routinely performed on citrated whole blood that, after recalcification, is activated using recombinant human tissue factor or kaolin, or is run native (without activation; Wiinberg and Kristensen, 2010). It is recommended that analysis of the blood samples be performed between 30 min and 2 h postsampling (Kol and Borjesson, 2010; Wiinberg and Kristensen, 2010).

Currently, 2 different analyzers are used worldwide: a thromboelastograph (**TEG**; TEG 5000, Haemonetics GmbH, Munich, Germany) and a rotational thromboelastometer (**ROTEM**; Tem International GmbH, Munich, Germany). The equipment differs in that the TEG has an oscillating cup, whereas the ROTEM has a rotating pin (Wiinberg and Kristensen, 2010). Both analyzers measure the same variables but using different terminology (Kol and Borjesson, 2010). The parameters assessed (in TEG terminology) consist of reaction time (**R**), which is the time in minutes from clot initiation until the first fibrin polymers are produced, clot formation time (**K**), the angle ($\alpha$) reflecting

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the kinetics of fibrin formation and crosslinking, and maximal clot strength (**MA**), which is displayed as the maximum amplitude in millimeters. Clot rigidity (**G**) is a modification of MA to physical units (Kol and Borjesson, 2010).

Comparison of the proprietary activation (kaolin for TEG; partial thromboplastin phospholipids for ROTEM) revealed significant differences between the representative single parameters; therefore, single parameter results are not directly comparable (Nielsen, 2007). This also applies to the different activation modes; therefore, separate validations are necessary for both analyzers and for the specific activation mode (Wiinberg and Kristensen, 2010).

Validations of thromboelastography with TEG and ROTEM in numerous species have been performed. Furthermore, studies using thromboelastography to investigate coagulopathies and other clinical conditions have been reported (Kol and Borjesson, 2010). Hypercoagulable states (e.g., due to DIC) could be identified in these studies.

Several bleeding disorders have been described in cattle (Bell, 2011), and hemostasis has been investigated. Reference intervals for coagulation parameters PT, APTT, TCT, and fibrinogen have been established for healthy cattle (Heuwieser et al., 1989), as has that for activated coagulation time (Riley and Lassen, 1979). Wittek et al. (2010) investigated the fibrin degradation products D-dimers in healthy cows, to assess increased fibrinolysis associated with coagulation, as seen with DIC.. The coagulation profile remained stable during pregnancy and parturition in the study by Gentry et al. (1979) but showed hypercoagulability in the periparturient period (3 d before and 2 d after), as revealed by shorter PT and partial thromboplastin times in the study by Heuwieser et al. (1990a). Fibrinogen values showed no significant differences between pregnant and nonpregnant cows (McSherry et al., 1970; Gentry et al., 1979; Schlerka and Baumgartner, 1994), unlike in the study of Heuwieser et al. (1990b), which reported significantly increased fibrinogen levels within the last 3 d before calving. Schlerka and Baumgartner (1994) found a significantly shorter APTT in pregnant compared with nonpregnant cows due to increased activity of factor IX.

Several of these coagulation studies in bovine species were coagulation factor-based, but thromboelastometry using ROTEM has recently been validated for cattle (Falco et al., 2012). The study population was mixed, including 14 male Holstein-Friesian calves, 15 Piedmontese cows, and 33 bulls (Holstein-Friesian, Charolais, and Piedmontese). Reference intervals were created for healthy adult cattle and calves; peripartal cows (1 mo before and after calving) were excluded (Falco et al., 2012). One study in calves has used thromboelastography (ROTEM) to investigate the hemostatic effect caused by low doses of dexamethasone (Borrelli et al., 2013).

The aim of this study was to validate thromboelastography for cows using kaolin-activated TEG analysis and to create reference intervals. An additional objective was to assess significant differences between different lactation states, including peripartal cows. Finally, we aimed to evaluate the stability of samples to allow submission of samples via courier, thus, making this test available to the general practitioner.

## MATERIALS AND METHODS

The samples were taken from cows from the university farm (Cambridge, UK), a 240-cow Holstein-Friesian commercial dairy unit, where cows were milked using automatic milking system (robots) and housed yearround in cubicles (freestalls). Cows were fed a mixed ration comprising mainly grass silage and a concentrate feed in the milking machine according to milk yield. Between July 2012 and March 2013, samples were made available as excess blood taken from cows undergoing metabolic profiling for health and production as part of the dairy herd health plan. This involved sampling of cows in early lactation (up to 30 d), at peak lactation (60 to 90 d), and past peak lactation. For this health check, 2 blood samples from each cow were taken from the coccygeal vein, consisting of one 10-mL serum tube and one 5-mL sodium citrate tube. Samples were chosen from 60 cows that were diagnosed as healthy according to the results from clinical examination, hematology, and biochemistry results.

The citrate blood samples, used for hematology, coagulation, and thromboelastography, were drawn first to minimize possible tissue factor contamination; correct filling of the tubes as well as evidence of clots in the sample were checked. Thromboelastography was carried out after hematology using the remaining citrate whole blood sample. Testing of coagulation times followed, performed with citrate plasma, obtained by centrifugation.

Thromboelastography was performed using the TEG 5000 (Haemonetics GmbH) exclusively by one of the authors (C.-C. S.). The age of the cows ranged from 2 to 10 yr with a mean and median of 5 yr. The cows were grouped according to their calving date (A = $\leq$30 d postcalving, B = 31–99 d postcalving, C $\geq$100 d postcalving). Twenty-four cows were classified into group A, 16 cows into group B, and 20 cows into group C. Of these 60 cows, 15 cows (4 from group B and 11 from group C) were pregnant with a mean of 88 d, median of 82 d, and ranging from 1 to 238 d (Table1).