**Original Article**

**TP, APTT and fibrinogen reference values in healthy beagle breed dogs**

Valores de referência de tempo de protrombina (TP), tempo de tromboplastina parcial ativada (TTPA) e fibrinogênio em cães hígidos da raça beagle

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**ABSTRACT**

Background: Hemostasis is a process of blood coagulation with the function of preventing hemorrhagic processes in the organism. The Coagulation Time (CT), Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Fibrinogen, Thrombin Time (TT), Increased Fibrin Degradation Products (IFDP) and D-Dimers are laboratory tests that can be used to evaluate the coagulation cascade. Objectives: The present study aimed to determine the reference values of PT, APTT and Fibrinogen by semi-automatic methodology with laboratorial kits.

Methods: Blood samples were collected from 22 healthy beagle dogs and immediately centrifuged and citrated plasma stored at -20 ºC for posterior analysis. PT, APTT and fibrinogen were measured using commercial kits in a semi-automatic coagulometer by the viscosity detection system.

Results: Mean values obtained were 6.0 ± 7.3 seconds for PT, 8.4 ± 16.9 seconds for APTT, and 10.2 ± 26.4 seconds for Fibrinogen.

Conclusion: It is concluded that the values obtained in this work can be used as reference for healthy Beagles.

**INTRODUCTION**

Hemostasis is the process of blood coagulation with the function of preventing hemorrhagic phenomena in the body, and can be subdivided into three phases: Primary, which shortly after vascular injury, local reflex vasoconstriction occurs, reducing blood flow so that the platelets may interact with the lesion site to form a platelet buffer; secondary, which is composed of a cascade of chemical processes subdivided into three pathways: intrinsic, extrinsic and common (coagulation cascade); and tertiary, in which the clot is degraded and fibrinolysis occurs (degradation of fibrin) and concomitant promotion of tissue repair (GARCIA-NAVARRO; PACHALY, 2005; VITAL, 2014).

The coagulation cascade (secondary hemostasis) is a set of chemical reactions that aim to polymerize the
fibrinogen in fibrin, avoiding blood loss (LOPES et al., 2005; BAKER, 2007). The cascade is divided into (i) the intrinsic pathway, that begins by the contact of the blood with a surface different from the inner wall of the vessel, and composed by the factors triggered in the following sequence: XII (Hageman), XI (Antihemophilic C), IX (Antihemophilic B), VIII (Antihemophilic A), X (Stuart-power); (ii) the extrinsic pathway, in which the prothrombin activating substance is generated in response to blood contact with the extravascular tissues by the activation of factor VII (Proconvertine) (HOFFMAN; MONROE, 2001). Both pathways culminate in the common pathway that begins with the activation of factor X and ends with the polymerization of fibrinogen in fibrin (LOPES et al., 2005) by the activation of thrombin.

The Coagulation Time (CT), Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Fibrinogen, Thrombin Time (TT), Increased Fibrin Degradation Products (IFDP), and D-Dimers are laboratory tests that can be used to evaluate the coagulation cascade, which may be altered due to thrombocytopenia, vasculitis, functional platelet defects, congenital coagulopathies (deficiencies of factor VII - hemophilia A or factor IX - hemophilia B) or acquired (liver diseases, vitamin K), snakebite, DIC (disseminated intravascular coagulation), von Willebrand disease and thrombosis which may be induced by renal, hepatic, hemoparasitosis, hereditary coagulopathies, neoplasias (BAKER, 2012; MARUYAMA et al., 2004).

The PT, APTT and Fibrinogen tests are the most accessible by commercial kits that can be performed by manual or semi-automatic (coagulometer) methodology, and through these correlated ones a diagnostic panel can be drawn on the hemostatic disorders when related to the thrombogram. PT evaluates the extrinsic and common pathway (factors VII, X, V, II and I) by the Macfarlane, Davis and Ratnoff methodology described in 1964 by providing tissue thromboplastin and calcium, that specifically activate the factor VII, which will lead to fibrin formation (MISCHKE; DIEDRICH; NOLTE, 2003).

The degree of PT prolongation is proportional to the severity of the deficiency of one or more factors involved in this pathway (MARTINS, 2011a). The APTT is used to monitor the intrinsic and common pathway (XII, XI, IX and VIII) and is indicated when there are hemorrhagic complaints and preoperative routines, by the methodology of Macfarlane, Davis and Ratnoff, that recommends the addition of cephalin, a phospholipid that together with calcium chloride promotes the formation of fibrin clot (CARLOS; FREITAS, 2007; MARTINS, 2011b). Fibrinogen plays two important roles: being an acute phase protein and also being the vital part of the common pathway for the materialization of coagulation. Using the Clauss methodology it is possible to measure the conversion rate of fibrinogen to fibrin in the presence of the excess of thrombin in a sensitive and precise way (HOFFMAN; MONROE, 2001; GOPEGUI et al., 2007; MARTINS, 2009).

The reagents used to determine PT, APTT and Fibrinogen are developed with bovine and rabbit serum matrices, validated in human plasma samples (LOPES et al., 2005), being fundamental the validation and establishment of reference values before the use for new species or in new apparatus of coagulometry. According to The International Federation of Clinical Chemistry and Laboratory of Medicine – Clinical and Laboratory Standards Institute (IFCC-CLSI), each laboratory should determine the reference values for its population considering its demographic characteristics associated to the characteristics and analytical methods used, and that can be based on at least 20 samples from the routine, eliminating up to two occurrences of outlier to respect the coefficient of variation of up to 2% (GEFFRE et al., 2011).

Thus, the aim of this study was to determine the reference values of PT, APTT and fibrinogen by semi-automatic methodology in a semi-automatic coagulometer (Quick Timer®) and laboratorial kits (Labtest) in Beagle breed dogs.

**MATERIAL AND METHODS**

Twenty-two beagle dogs (16 females and 6 males) were selected. Among them, 18 adults (male x female) aged between one and seven years, and four young (male x female), aged less than one year, from the – (LOCAL). All animals were found to be healthy based on clinical and laboratory evaluations (blood count, creatinine and alanine aminotransferase). Ethics approval with permission to conduct the study was received by the Ethics Committee on the Use of Animals (Protocol n°. 003549/2019, 11th April 2019).

For this evaluation, 3 mL of whole blood were collected by jugular venipuncture in 3,2% sodium citrate tubes, immediately centrifuged for 5 minutes at 1000g to obtain plasma. The citrated plasmas were aliquoted in microtubes and stored (-20°C) until the moment of the analysis, where they were thawed until reach controlled laboratory temperature (23°C).

Commercial kits used were PT Hemostasis (Ref. 501, Labtest Diagnóstica, Brazil), APTT Hemostasis (Ref. 502, Labtest Diagnóstica, Brazil) and Fibrinogen (Ref. 506, Labtest Diagnóstica, Brazil) following the instructions of use recommended by the manufacturer. The results of the hemostatic variables were obtained by means of a semi-automatic coagulometer Quick Timer® (Drake Technologies, Brazil) by the chronometric method of clot evaluation.

All the results were submitted to the normality test of Cramér-von Mises (p ≤ 0.05), and for better presentation, were described in median (amplitude). The results were presented in a single group and divided into two categories (sex and age). The mean values categorized by sex and age, with homosedasticity assessed by the F-Test (p≤0.05), were compared using
the t-Test or Wilcoxon Mann-Whitney Test to determine if there was an obligation to maintain the division of results found, with degree confidence for both tests of 95%. The present experiment used the software R (R Core Team, Austria) for the statistical procedures described.

RESULTS

The results are organized as follows, Table 1 shows the values of all dogs sampled (n=22) for PT, APTT, and fibrinogen. Table 2 shows the results referring to the variance values of hemostatic parameters categorized by sex and age.

Table 3 shows the hemostatic parameters according to sex (females n=16, males n=6) and, as can be verified, the medians for all values did not present significant statistical difference. In table 4, hemostatic values are stratified by the age group of the dogs sampled (adults n=18, young n=4), and, as can be verified, there was no significant difference in the comparison between adults and young.

Considering the non-significant statistical results, when comparing the sources of sex and age variation, we chose an evaluation of the results that did not consider the sex and age variables, but the values obtained here compared to those cited in the literature as reference values. Thus, when comparing the values obtained in this study with those of Maecelbergh; Acierno (2008) and Geffre et al. (2011), no significant differences were observed in the Wilcoxon test, as seen in table 5.

Table 1. Median and Amplitude values for PT, APTT and Fibrinogen in healthy beagle dogs (n=22) from LOCAL.

| Hemostatic parameter                  | Median (Amplitude) | Normality Cramér-von Mises (p≤0.05) | CV (%) |
|---------------------------------------|--------------------|--------------------------------------|--------|
| **Prothrombin Time (PT)**             |                    |                                      |        |
| Seconds                               | 6.25 (6 – 7.30)    | 0.003                                | 6.02   |
| INRa                                  | 0.52 (0.5 – 0.61)  | 0.001                                | 5.94   |
| **Activated Partial Thromboplastin Time (APTT)** |                   |                                      |        |
| Seconds                               | 12.7 (8.4 – 16.9)  | 0.898                                | 17.29  |
| **Fibrinogen**                        |                    |                                      |        |
| Seconds                               | 20.7 (10.2 – 26.4) | 0.472                                | 17.84  |
| g/L                                   | 2.5 (1.8 – 8.4)    | 2.077 x 10^{-7}                     | 59.11  |

aINR: International Normalized Ratio; bProthrombin activity index.

Table 2. P values obtained in F-Test of the comparison of the variances in relation to the comparison of the categories of experimental units tested (female vs. male, adults vs. young) for hemostasis test of Prothrombin, Activated partial thromboplastin and Fibrinogen.

| Hemostatic tests (UI) | Categories | F-Test1 (p≤ 0.05) |
|-----------------------|------------|-------------------|
| Prothrombin (sec)     | Female     | 0.018             |
|                       | Adult      | 0.462             |
|                       | Young      | 0.516             |
| Prothrombin (INR)     | Female     | 0.011             |
|                       | Adult      | 0.013             |
|                       | Young      | 0.146             |
| Prothrombin (I)       | Female     | 0.019             |
|                       | Adult      | 0.212 x 10⁻⁶     |
|                       | Young      | 0.340             |
| Activated partial thromboplastin (seg) | Female     | 0.011             |
|                       | Adult      | 3.19 x 10⁻⁷      |
|                       | Young      | 0.003             |
| Fibrinogen (seg)      | Female     | 0.44 x 10⁻¹⁵     |
|                       | Adult      | 0.44 x 10⁻¹⁵     |
| Fibrinogen (g/L)      | Female     | 0.340             |
|                       | Adult      | 3.19 x 10⁻⁷      |
|                       | Young      | 0.003             |

1. Parametric means with significant variances (p≤0.05) were statistically compared by the Welch t-test (p≤0.05). Parametric means with non-significant variances (p≤0.05) were compared by the t-test (p≤0.05). INR: International normalized ratio. I: Prothrombin activity index.
### Table 3. Values of hemostatic parameters (Prothrombin, Activated partial thromboplastin and Fibrinogen) of beagle dogs (n=22) categorized by sex.

| Beagle dogs | Median (Amplitude) | Normality Cramér-von Mises (p≤0.05) | Wilcoxon Test (p≤0.05) |
|-------------|--------------------|--------------------------------------|-----------------------|
| Test        | UI     | Sex  |               |                      |                       |
| PT          | Sec    | ♀    | 6.25 (6 – 7)  | 0.201                | 0.8796                |
|             |        | ♂    | 6.2 (6 – 7.3) | 0.052                |                       |
| INR         | ♀      | 0.52 (0.5 – 0.58) | 0.125         | 0.8793                |
|             | ♂      | 0.51 (0.5 – 0.61) | 0.05         |                       |
| I           | ♀      | 0.445 (0.42 – 0.51) | 0.080       | 0.7892                |
|             | ♂      | 0.44 (0.42 – 0.54) | 0.062       |                       |
| APTT        | Sec    | ♀    | 13 (9.3 – 16.9) | 0.941                | 0.4503*               |
|             |        | ♂    | 12.45 (8.4 – 15.3) | 0.864          |                       |
| Fibrinogen  | Sec    | ♀    | 20.7 (7.4 – 26.4) | 0.032                | 0.6846                |
|             |        | ♂    | 19.2 (15.9 – 23.60) | 0.441          |                       |
|             |        | ♀    | 2.5 (1.8 – 8.4) | 3.973 X 10^-6 | 0.9117                |
|             |        | ♂    | 2.65 (2 – 3.30) | 0.603                |                       |

*The mean values were compared through the t-test (p≤0.05), since the data distribution was considered parametric by the Cramér-von Mises test (p≤0.05). INR: International normalized ratio. I: Prothrombin activity index.

### Table 4. Values of hemostatic parameters (Prothrombin, Activated partial thromboplastin and Fibrinogen) of beagle dogs (n=22) categorized by age.

| Beagle dogs | Median (Amplitude) | Normality Cramér-von Mises (p≤0.05) | Wilcoxon Test (p≤0.05) |
|-------------|--------------------|--------------------------------------|-----------------------|
| Test        | UI     | Age  |               |                      |                       |
| PT          | Sec    | Adults | 6.3 (6 – 7.10) | 0.057                | 0.8056                |
|             |        | Youngs| 6.35 (6 – 7.3) | 1.941 X 10^-8       |                       |
| INR         | ♀      | Adults | 0.52 (0.5 – 0.59) | 0.03         | 0.3371                |
|             | ♂      | Adults | 0.53 (0.5 – 0.61) | 0.127        |                       |
|             |        | Youngs| 0.45 (0.42 – 0.52) | 0.014       | 0.8052                |
|             |        | Youngs| 0.452 (0.42 – 0.54) | 0.196       |                       |
| I           | ♀      | Adults | 12.55 (8.4 – 16.9) | 0.967       | 0.3589*               |
|             | ♂      | Adults | 12.95 (11.7 – 15.3) | 0.747       |                       |
| APTT        | Sec    | Adults | 20.7 (10.2 – 26.4) | 0.802       | 0.1561*               |
|             |        | Youngs| 17.7 (16 – 21) | 0.223                |                       |
| Fibrinogen  | Sec    | Adults | 2.5 (1.8 – 8.4) | 2.213 X 10^-6 | 0.2889                |
|             |        | Youngs| 2.30 (2.65 – 3.10) | 0.408       |                       |

*The mean values were compared through the Welch t-test (p≤0.05), since the data distribution was considered parametric by the Cramér-von Mises Test (p≤0.05). INR: International normalized ratio. I: Prothrombin activity index.
Table 5. Comparison of means of Prothrombin Time (PT), Partial Activated Thromboplastin Time (APTT) and Fibrinogen of Beagle dogs (n=22) with averages described in the literature. \textbf{Wilcoxon (p ≤ 0.05)}.  

| PARAMETER          | EXPERIMENT (RESULTS) | LITERATURE | WILCOXON TEST (p ≤ 0.05) |
|--------------------|-----------------------|------------|--------------------------|
|                    |                       | Papper     | n                         | Results                  |
|                   |                       | I          | 40                        | 6.87 ± 1.4 (4.07 – 9.67) | 0.0001                   |
|                   |                       | II         | 35                        | 7.5 – 10                  | 3.851 x 10⁻⁵              |
|                   |                       | III        | 75                        | 7.6                       | 3.851 x 10⁻⁵              |
|                   |                       | IV         | 56                        | 5.7 – 8.1                 | 8.846 x 10⁻⁵              |
|                   |                       | V          | 20                        | (11.03 – 13.05)           | 3.851 x 10⁻⁵              |
|                   |                       | VI         | 9                         | 8.0 ± 0.8                 | 3.851 x 10⁻⁵              |
|                   |                       |            |                           |                           |                          |
|                   |                       | I          | 40                        | 15.1 ± 1.6 (11.9 – 18.3)  | 0.01                      |
|                   |                       | II         | 35                        | 11 – 14                   | 0.613                     |
|                   |                       | III        | 75                        | 15                       | 0.001                     |
|                   |                       | IV         | 56                        | 10 – 14.3                 | 0.178                     |
|                   |                       | V          | 20                        | 18                       | 4.291 x 10⁻⁵              |
|                   |                       | VI         | 9                         | 16.9 ± 2.9                | 9.556 x 10⁻⁵              |
|                   |                       | VII        | 30                        | 11.5 ± 1.25               | 0.016                     |
|                   |                       | I          | 35                        | 1.50 – 2.65               | 4.271 x 10⁻⁵              |
|                   |                       | II         | 35                        | 1.3 – 3.1                 | 4.271 x 10⁻⁵              |
|                   |                       | IV         | 56                        | 234.52 (186.14 – 234.52)  | 4.271 x 10⁻⁵              |
|                   |                       | V          | 20                        | 1.93 ± 0.41               | 4.271 x 10⁻⁵              |
|                   |                       | VI         | 9                         |                           |                           |

DISCUSSION

The time obtained in this trial for the PT was less prolonged than that reported by Rizzo et al. (2008), who used manual methodology. It is quite possible that such a difference may be associated with the use of the semi-automatic method in this study. On the other hand, Lopes et al. (2005) also used the manual methodology and obtained the mean values of PT closer to those of the present study, yet, with higher values dispersion (4.07 to 9.67 seconds) which suggests a wide variation of results with the use of manual methods.

Maeckelbergh; Acierno (2008) and Grochowsky et al. (2014) obtained longer prothrombin times in a semi-automatic coagulometer compared to the present study. Similar situation was the comparison with the results described by Romão et al. (2013) in citrated plasma of dogs, before the administration of corticosteroids, in which they report a mean value of PT of 11.8 seconds using an automatic methodology. Such a value is much higher than that found in this study, which may be related to the determination in an automatic coagulometer that does not suffer any influence of the operator as in the manual or semi-automatic methodology. The values found in percentage of reaction (%) and Internacional Normalized Ratio – INR (i), for PT, corroborated the results described by Mischke (2011).

Romão et al. (2013) observed higher values of APTT with greater amplitude (15.75 – 19.21 sec). In the case of this test, however, the value found for Beagles was 12.7 sec (8.4 – 16.9 sec), close to the amplitude time reported by Maeckelbergh; Acierno (2008) (11-14 sec). On the other hand, Grochowsky et al. (2014) reported much longer APTT values using automatic methodology. Such divergences in values highlight the need to construct a specific value curve for each method (manual, semi-automatic and automatic) of hemostatic analysis.

When compared with results of Lopes et al. (2005), our means values obtained (PT and APPT) were different (p = 0.0001) to the ones observed in our study, 12.7 (8.4 to 16.9 sec), which may be associated to the difference to the kit and methodology used between the studies.

A similar situation was found in relation to the study by Rizzo et al. (2008) (PT: p = 3.851 x 10⁻⁵ and APPT: p = 0.001). Therefore, it can be affirmed that the manual methodology of obtaining the APTT provides results almost always discordant to those obtained by semi-automatic tests.

Compared to the values of APTT obtained automatically, the means obtained in this study were similar to those reported by Evans; Flynn (1992), who used two semi-automatic coagulometers and observed that there were differences between them, and it is, therefore, of paramount importance to consider the choice and determination of reference value for the apparatus as an analytical factor. Geffré et al. (2011) obtained results close to the present assay, and stated that the hemostasis
tests are not free of the methodological, instrumental and specificity influence of the reagent.

Comparing the mean values of fibrinogen described by Maexkelbergh; Acierno (2008), Geffré et al. (2011), Romão et al. (2013), and Grochowsky et al. (2014), it is verified that there was proximity to those obtained in this study. However, there was a greater dispersion of the values obtained (maximum and minimum values) in the cases of the mentioned authors. In all cited references there was no expression of the fibrinogen time (seconds), only the concentration to promote coagulation (g/dL).

As reported by Maexkelbergh; Acierno (2008), the values of fibrinogen for hemostasis are confounded with its role as a phase-acute protein, a situation that is reflected in the research studies, which reports only the values of PT and APTT, excluding dosage of fibrinogen, used for polymerization of fibrin.

**CONCLUSIONS**

Under the conditions in which this study was performed, we concluded that there were no significant differences between sex and age in the laboratory evaluation of PT, TTPA, and Fibrinogen for the purpose of coagulation tests in Beagle dogs, yet, it is important to consider the type of methodology used in the mentioned tests. The values of PT, APTT, and Fibrinogen obtained in the present study can be used as a reference for healthy Beagles, by the semi-automatic methodology.

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