Journal of the American Association for Laboratory Animal Science
Copyright 2016
by the American Association for Laboratory Animal Science

Vol 55, No 3
May 2016
Pages 252–259

Coagulation Biomarkers in Healthy Chinese-Origin Rhesus Macaques (*Macaca mulatta*)

Galit H Frydman,[1,2,5,*] Pavan K Bendapudi,[3,5] Robert P Marini,[1] Charles R Vanderburg,[4,5] Ronald G Tompkins,[2,5] and James G Fox[1]

Rhesus macaques (*Macaca mulatta*) are a common model for the study of human biology and disease. To manage coagulopathies in these animals and to study their clotting changes, the ability to measure coagulation biomarkers is necessary. Currently, few options for coagulation testing in NHP are commercially available. In this study, assays for 4 coagulation biomarkers—D-dimer, antithrombin III, protein C, and soluble P-selectin—were developed and optimized for rhesus macaques. Whole blood was collected from 28 healthy Chinese-origin rhesus macaques (11 male; 17 female) ranging in age from 5 to 20 y. Coagulation biomarkers were measured by using bead-based sandwich ELISA technology. The ranges (mean ± 90% confidence interval) for these biomarkers were: antithrombin III, 124.2 to 133.4 μg/mL; protein C, 3.2 to 3.6 μg/mL; D-dimer, 110.3 to 161.3 ng/mL; soluble P-selectin, 0.12 to 0.14 ng/10^6 platelets. These reference values did not differ significantly according to sex or age. These new assays for coagulation biomarkers in rhesus macaques will facilitate the evaluation of in vivo hemostasis.

Abbreviations: CV, coefficient of variation; soluble P-selectin, sCD62P.

Coagulopathies are a common feature of many human diseases. Acquired coagulopathies represent an important cause of morbidity and mortality across a spectrum of disorders, including trauma, malignant neoplasms, liver disease, and systemic autoimmune and inflammatory states. NHP are frequently used as models for human disease. In particular, rhesus macaques (*Macaca mulatta*) have a 92.7% genetic homology to humans, making this species a commonly used model in the study of transplantation medicine, as well as numerous infectious agents, including SIV, Ebola, dengue, malaria, hantavirus, plague, and rickettsial diseases.[7,11,14,18,23,26,30,31,34,35]

Many of these conditions are associated with bleeding and clotting complications. When assessing bleeding diatheses, in vitro coagulation assays, such as prothrombin time and activated partial thromboplastin time, have been the backbone of clinical testing. Although these in vitro coagulation assays and measurements of coagulation factors are easy to use and interpret, they often fail to reflect in vivo processes and do not capture the true risk of bleeding or clotting.[12] Various coagulation proteins, including D-dimer and antithrombin III, are increasingly being evaluated as biomarkers that might more accurately reflect the risk of hemostatic complications.[5,17,20,25]

The measurements of coagulation biomarkers are frequently limited to specialty clinical coagulation laboratories, logistically and financially hindering their use. Another common obstacle to measuring coagulation parameters in NHP is the lack of species-specific reagents. Here we describe the development of a bead-based ELISA assay to determine the normal ranges of 4 coagulation biomarkers in a population of healthy rhesus macaques: D-dimer, antithrombin III, protein C, and soluble P-selectin (sCD62P).

Received: 03 Jul 2015. Revision requested: 17 Aug 2015. Accepted: 13 Oct 2015.
[1]Division of Comparative Medicine and Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts; [2]Division of Surgery, Science, and Bioengineering and [3]Division of Hematology, Massachusetts General Hospital, Boston, Massachusetts; [4]Advanced Tissue Resource Center, Institute for Neurodegenerative Disease, Massachusetts General Hospital, Charlestown, Massachusetts; and [5]Harvard Medical School, Boston, Massachusetts.
[*]Corresponding author. Email: gfrydman@mit.edu.

Materials and Methods

Animals and husbandry. A population of 28 healthy, Chinese-origin rhesus macaques (age, 5 to 20 y; female, 17; male, 11) was used in clinical coagulation testing. All study animals were indoor-housed, either singly or paired, in an AAALAC-accredited facility. Commercial primate chow (Lab Diet 5038, PMI Nutrition International, St Louis, MO) was fed twice daily. Environmental enrichment was provided daily in the form of toys, videos, fruits, vegetables, and other treats. While in quarantine, macaques were screened for endoparasites, *Salmonella* spp., *Shigella* spp., other enteric pathogens, tuberculosis, and a battery of viral agents including simian retrovirus, *Macacine herpesvirus* type 1 (B virus), simian T-lymphotrophic virus 1, measles virus, and SIV. With the exception of infrequent positive measles antibody titers, none of the macaques was positive for these agents. Each macaque is annually tested and confirmed negative for enteric pathogens and is semiannually tested for tuberculosis, fecal endoparasites, and B virus. All of the macaques were on IACUC-approved experimental protocols.

Blood collection and processing. All macaques were sedated with either ketamine (10 mg/kg IM) or tiletamine–zolazepam (5 mg/kg IM). Blood was collected from the femoral vein into two 2.9-mL sodium citrate (3.2%) and one 1.2-mL EDTA vacuum phlebotomy tubes (Sarstedt, Nümbrecht, Germany). The first sodium citrate tube was discarded to minimize the chance that endothelial and platelet activation from the initial venipuncture would result in spurious biomarker readings (that is, a '2-tube technique' was used).[21] Platelet-poor plasma was prepared within 4 h of the blood draw and was collected from the citrate tubes by 2 sequential centrifugations at 1700 x *g* for 10 min. The platelet-depleted plasma was then separated into 200-μL aliquots and stored at –80 °C until analyzed. A CBC analysis was performed inhouse (Hemavet 950 FS, Drew Scientific, Oxford,

CT) to obtain the total platelet count. Any platelet count that was below 250×10^6/mL was reviewed manually by using a peripheral blood smear. If a sample was noted to have platelet clumps on the peripheral smear, it was discarded from analysis.

Sequence homology. To determine the level of sequence homology between the human and rhesus macaque coagulation biomarker targets in this study, a BLAST analysis on Uniprot (http://www.uniprot.org) was performed.[4] Because D-dimer is a multimer composed of the fibrinogen β chain and γ chain monomers, the sequences for these monomers were used in the homology analysis of D-dimer.

Measurement of coagulation biomarkers. A magnetic bead-based sandwich ELISA format was used to obtain quantitative outputs for coagulation biomarkers. Antibodies and recombinant proteins for D-dimer, antithrombin III, protein C, and soluble sCD62P were purchased from R and D Systems (Minneapolis, MN), Novus Biologicals (Littleton, CO), Haematologic Technologies (Essex Junction, VT), Affinity Biologicals (ON, Canada), and Abcam (Cambridge, UK), respectively. Human plasma was used in assay development due to the lack of commercially available macaque plasma. Normal and abnormal human plasma were purchased from commercial sources (Enzyme Research Laboratories, South Bend, IN). Magnetic bead coupling and confirmation of bead coupling were performed according to manufacturer's instructions (Luminex, Austin, TX). When possible, multiple targets were analyzed in the same assay utilizing established multiplexing methods.

The assays were developed and optimized according to standard protocols from the bead manufacturer (Luminex). Samples were read by using a Flexmap 3D (Luminex). The standard curve was generated by using 5-parametric-curve fitting with platform-specific software (xPONENT Software Solutions, Luminex, Austin, TX). Control samples were run in duplicate on each plate for quality control. Plasma was diluted to the optimal concentration to be read within the range of the standard curve. The inter- and intraassay variability for each of the targets is reported in Table 1.

Statistical analysis. For each biomarker, the intraassay coefficient of variation (CV) was calculated based on samples run in duplicate on each plate. For each biomarker, the interassay CV was calculated based on the same clinical sample, run in duplicate on at least 3 plates on 3 different days. The highest and lowest values of each biomarker were removed from analysis. Histograms were created for each of the biomarkers to assess for normality.[15] The Horn algorithm with Turkey interquartile fences was used to identify outliers associated with the upper and lower extremities.[15] All the biomarkers exhibited a Gaussian distribution, confirmed by the Shapiro–Wilk test ($P < 0.05$); therefore, analysis was performed using parametric methods.[15] The mean and 90% confidence intervals were calculated for all determinations and separately for both sexes. Independent sample 2-tailed student *t* tests assuming equal variance were performed to identify significant differences between the male and female groups for each biomarker. The Pearson correlation coefficient between age and each biomarker was calculated. Statistical significance was defined as a *P* value of 0.05 or less. All statistical analysis was performed by using GraphPad Prism (GraphPad Software, San Diego, CA), StatPlus (AnalystSoft, Walnut, CA), and Excel (Microsoft, Redmond, WA).

Results

Sequence homology. The female rhesus macaques had a mean age of 11 ± 5.4 y, whereas male macaques had a mean age of 8 ± 3.7 y. Sequence homology between the human (*Homo sapiens*) and rhesus macaque (*M. mulatta*) forms of the biomarkers for the coagulation targets was confirmed (Table 1). According to these results, the antibodies we used in this study are likely able to accurately detect rhesus macaque targets, despite being originally raised against human antigens.

Antithrombin III. The assay had an average R^2 of 0.964 on the basis of an 8-point standard curve, with the minimal detectable level of antithrombin III being 0.13 ng/mL (Figure 1 A, Table 2). There were no outliers (according to the Horn algorithm) among the 25 samples, and the Shapiro–Wilk test *P* value of 0.64 signified a normal distribution (Table 3, Figures 2 A and 3 A). The male plasma antithrombin III concentration ranged from 109.9 to 147.1 µg/mL, whereas that for female macaques was 117.8 to 140.2 µg/mL (Table 4, Figure 4 A). There was no correlation between the antithrombin III level and age for either male or female macaques (Pearson r = –0.04) or significant difference between sexes ($P = 0.939$; Table 4). According to these results, the expected normal range (mean ± 90% confidence interval) of antithrombin III in rhesus macaque is 124.2 to 133.4 µg/mL (Table 3).

D-dimer. The assay had an average R^2 of 0.988 on the basis of an 8-point standard curve, with the minimal detectable level of D-dimer being 2.29 ng/mL (Figure 1 B, Table 2). Among the 22 samples evaluated, 2 outliers were discarded according to the Horn algorithm. After the removal of these outliers, the Shapiro–Wilk test yielded a *P* value of 0.45, signifying normal distribution (Table 3, Figures 2 B and 3 B). The male plasma D-dimer concentration ranged from 48.2 to 181.2 ng/mL, whereas that for female macaques was 95.9 to 203.9 ng/mL (Table 4, Figure 4 B). There was no correlation between the D-dimer level and age for either male or female macaques (Pearson r = 0.009) or significant difference between sexes ($P = 0.209$; Table 4). According to these results, the expected normal range (mean ± 90% confidence interval) of D-dimer in the rhesus macaque is 110.3 to 161.3 ng/mL (Table 3).

Protein C. The assay had an average R^2 of 0.976 on the basis of an 8-point standard curve, with the minimal detectable level of protein C being 0.13 ng/mL (Figure 1 C, Table 2). There were no outliers according to the Horn algorithm among the 25 samples, and the Shapiro–Wilk *P* value of 0.48 indicated normal distribution (Table 3, Figure 2 C and 3 C). The male protein C concentration ranged from 2.8 to 3.9 µg/mL, whereas that for female macaques was 2.7 to 4.2 µg/mL (Table 4, Figure 4 C). There was no correlation between protein C level and age for either male or female macaques (Pearson r = 0.031) or significant difference between sexes ($P = 0.727$; Table 4). According to these results, the expected normal range (mean ± 90% confidence interval) of protein C in rhesus macaques is 3.2 to 3.6 µg/mL (Table 3).

Soluble P-selectin. sCD62P is released into the plasma as a result of platelet activation. Preanalytical variables are of the utmost importance when assessing for markers of platelet activation, because CD62P can be expressed by the platelet and released as a soluble factor within seconds and can be completely lost from the platelet surface within 2 h.[22] The platelet-depleted plasma must be prepared as soon as possible, within 2 to 4 h of blood drawing if possible, and the anticoagulant used is also a significant variable.[1,19] Once the platelet-depleted plasma is prepared from whole blood, there is no other source of sCD62P, but the sample should be analyzed promptly or frozen at –80 °C to prevent protein degradation.[33] During the analysis of sCD62P, the platelet count of a subject must be considered; for example, an otherwise normal sCD62P concentration in a subject with a normal platelet count would

Table 1. Sequence comparison of coagulation biomarker genes

Target	Pathway	Protein length (no. of amino acids)		%Homology between proteins
		Homo sapiens	*Macaca mulatta*	
Antithrombin III	Anticoagulation	464	464	97
Fibrinogen β chain[a]	Coagulation and fibrinolysis	491	491	96
Fibrinogen γ chain[a]	Coagulation and fibrinolysis	453	453	96
Protein C	Anticoagulation	461	462	95
sCD62P	Platelet activation	830	829[b]	95

[a]Used as representative for D-dimer sequence homology
[b]Uncharacterized protein

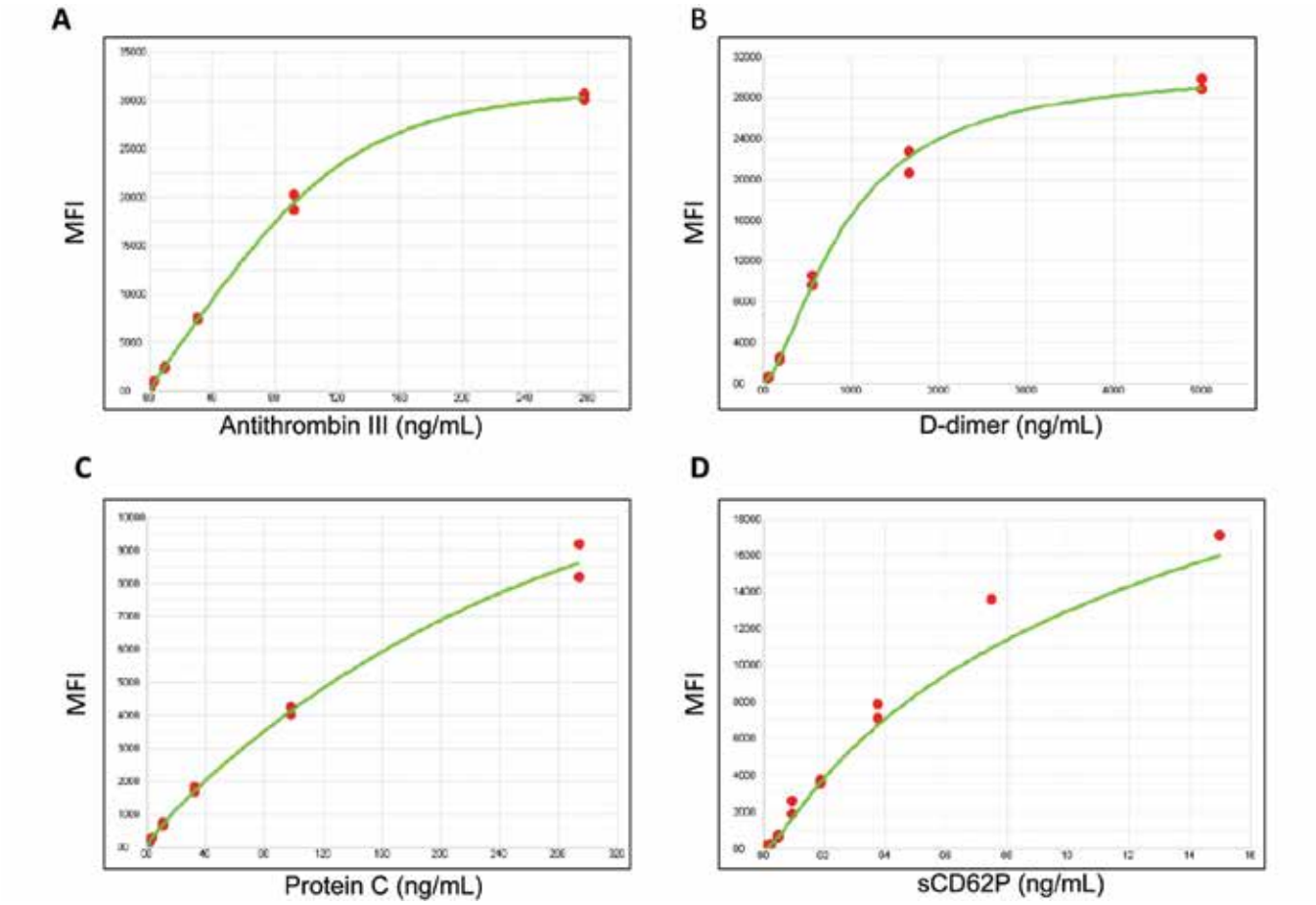

Figure 1. Representative standard curves of coagulation biomarkers in rhesus macaques. The standard curve was generated by using 5-parametric-curve fitting in platform-specific software. (A) Antithrombin III standard curve, $R^2 = 0.98$. (B) D-dimer standard curve, $R^2 = 0.97$. (C) Protein C standard curve, $R^2 = 0.99$. (D) sCD62P standard curve, $R^2 = 0.97$. MFI, mean fluorescence intensity.

indicated elevated sCD62P levels in a subject with a decreased platelet count.

The assay had an average R^2 of 0.967 on the basis of an 8-point standard curve, with the minimal detectable level of soluble sCD62P being 0.12 ng/mL (Figure 1 D, Table 2). A single outlier (according to the Horn algorithm) was discarded to yield a total of 24 samples, which were associated with a Shapiro–Wilk *P* of 0.50, signifying normal distribution (Table 3, Figures 2 D and 3 D). The male plasma sCD62P concentration ranged from 0.11 to 0.19 ng/10^6 platelets (Table 5), and that for female macaques was 0.11 to 0.15 ng/10^6 platelets (Tables 4 and 6, Figure 4 D). There was no correlation between sCD62P concentration and age for either male or female macaques (Pearson r = –0.253) or significant difference between sexes (*P* = 0.194; Table 4). According to these results, the expected normal range (mean ± 90% confidence interval) of sCD62P in rhesus macaques is 0.12 to 0.14 ng/10^6 platelets (Table 3).

Table 2. Assay parameters and variance

	Average fit of standard curve[a]	Average intraassay CV (%)	Average interassay CV (%)
Antithrobin III	0.964	7.59	11.56
D-dimer	0.988	12.24	16.64
protein C	0.976	6.43	25.55
sCD62P	0.967	9.04	2.12

[a]Calculated based on the mean R^2 value of the standard curve according to 5-parametric-curve fitting of all sample plates run.

Table 3. Biomarker ranges and descriptive statistics in a population of healthy adult rhesus macaques

	Antithrombin III (µg /mL)	D-dimer (ng /mL)	protein C (µg /mL)	sCD62P (ng/ 10^6 platelets)
Sample size	25	20	25	24
Mean	128.8	135.8	3.40	0.13
90% Confidence interval	4.6	25.5	0.22	0.01
Minimum	103.7	39.8	2.33	0.09
Maximum	156.6	241.1	4.84	0.21
Skewness[a]	−0.06	0.03	0.43	0.83
Kurtosis[a]	−0.42	−0.72	0.04	0.80
25% Quartile	124.1	100.2	2.92	0.11
75% Quartile	137.7	171.4	3.78	0.15
Interquartile range	13.6	71.2	0.86	0.04
Shapiro–Wilks *P*	0.64	0.45	0.48	0.50

[a] Fisher-Pearson coefficient

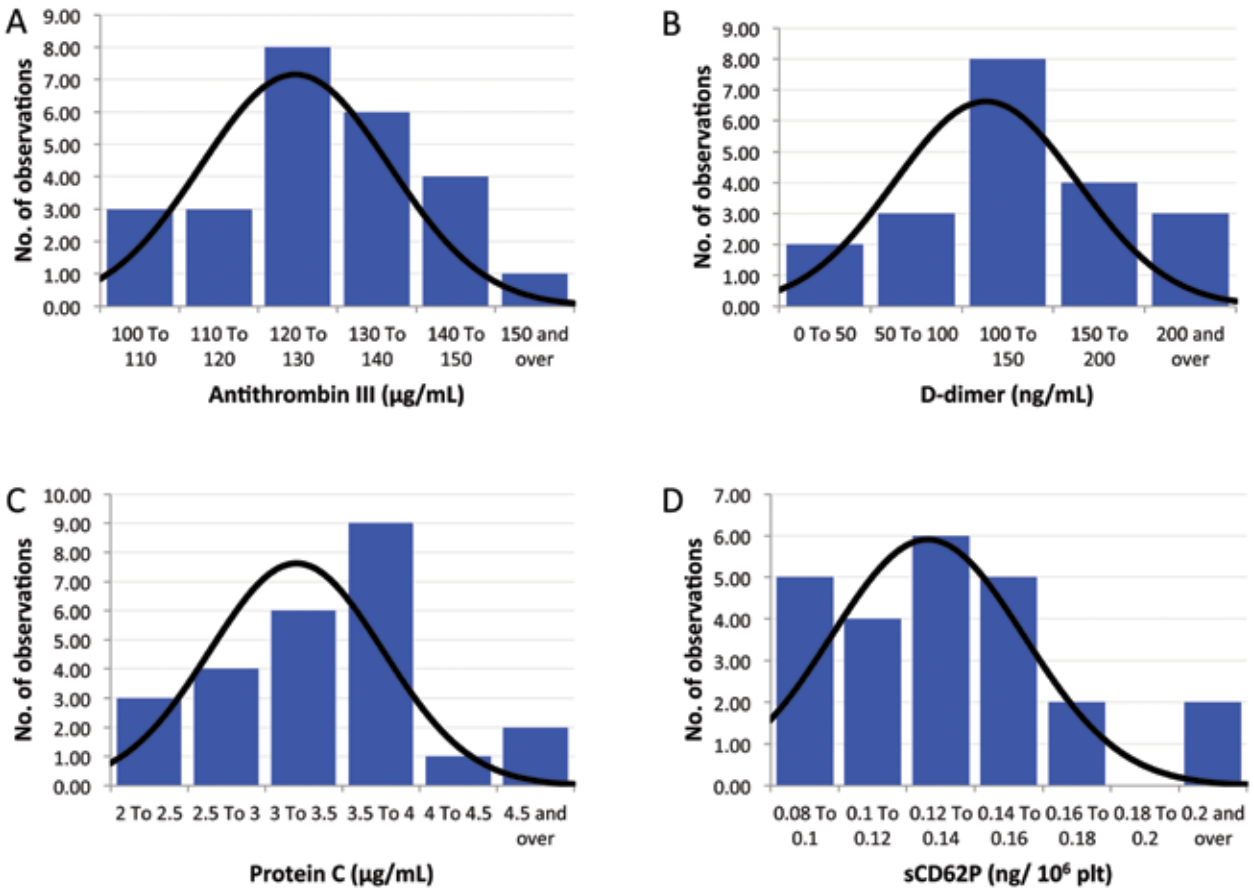

Figure 2. Representative histograms and overlaid normal curves. (A) Antithrombin III. (B) D-dimer. (C) Protein C. (D) sCD62P.

Discussion

Here we describe the development of bead-based sandwich ELISA assays for 4 clinically relevant coagulation biomarkers in Chinese-origin rhesus macaques (*M. mulatta*) and report ranges for these molecules in this healthy population. These biomarkers can provide valuable information about a subject's coagulation state. There are 4 main arms of the hemostatic system: coagulation, anticoagulation, fibrinolysis, and platelet activation (Figure 5). D-dimer is a fibrin degradation product that forms when crosslinked fibrin is cleaved by plasmin.[36] Circulating D-dimer is therefore an indicator of fibrinolytic activity, and an elevated D-dimer can be suggestive of ongoing abnormal clot turnover or a primary hyperfibrinolytic state. Antithrombin III is a natural anticoagulant that is synthesized in the liver. Acting as a serine protease inhibitor, antithrombin III binds and inhibits a number of coagulation factors, including factors II and X.[29] Protein C is a vitamin-K–dependent factor which, when activated, also functions as a natural anticoagulant; protein C also has cytoprotective and anti-inflammatory roles.[13,24] P-selectin (CD62P) is a cell-adhesion molecule normally present in platelet α-granules. After platelet activation, CD62P translocates to the cell surface, and some of the translocated protein is released into the plasma in a soluble form. Although CD62P can originate from the Weibel–Palade bodies of activated endothelial cells,

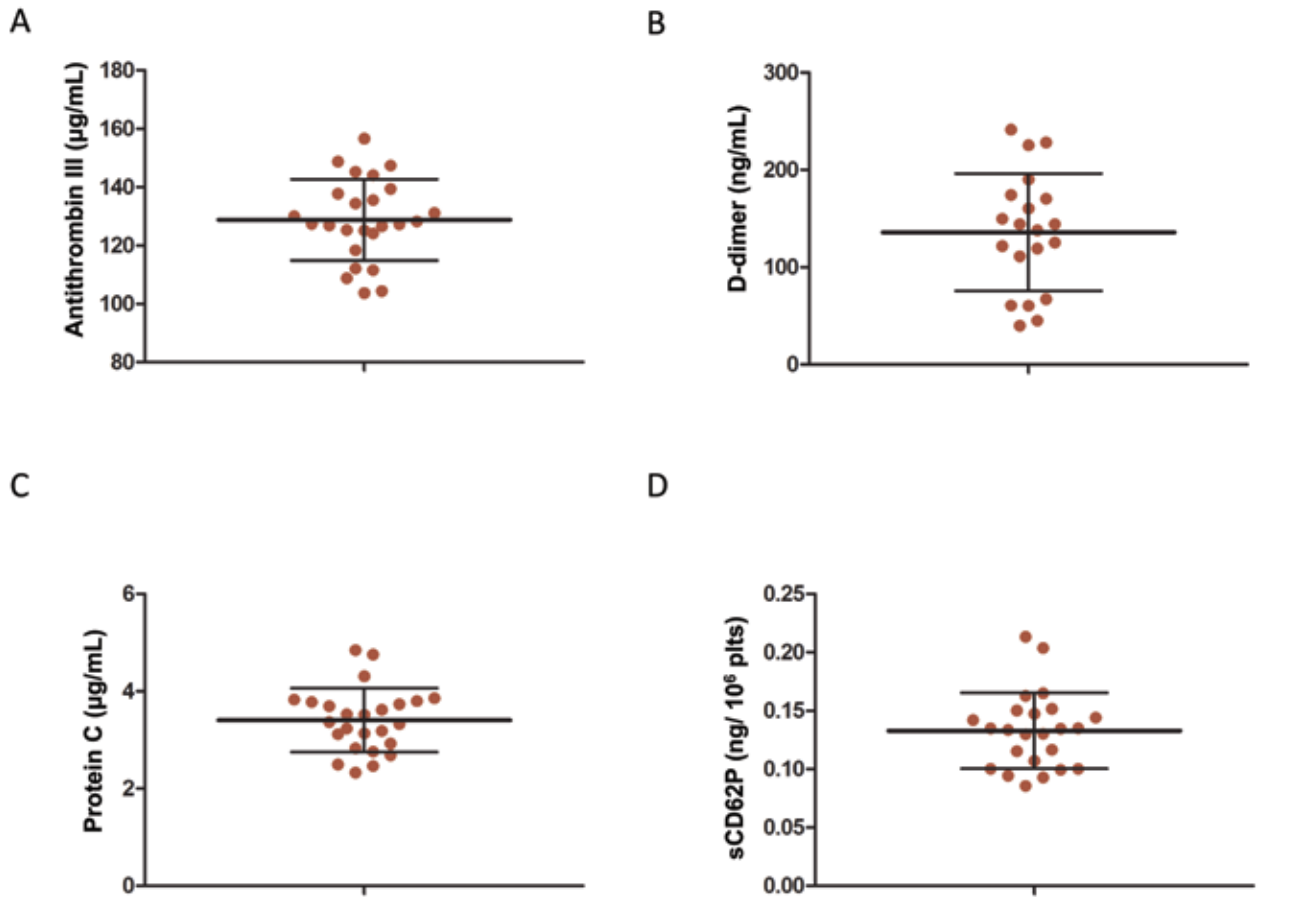

Figure 3. Scatter plots of each biomarker with the mean (middle horizontal bar) and standard deviation error bars for rhesus macaques. (A) Antithrombin III (mean ± 1 SD), 128.8 ± 13.9 µg/mL. (B) D-dimer, 136.4 ± 69.3 ng/mL. (C) Protein C, 3.4 ± 0.7 µg/mL. (D) sCD62P, 0.13 ± 0.04 ng/10^6 platelets.

Table 4. Biomarker comparison according to sex and age in a population of healthy adult rhesus macaques

	Male macaques		Female macaques			
	n	Mean ± 1 SD	*n*	Mean ± 1 SD	R^{2a}	*P*[b]
Antithrombin III (µg/mL)	9	128.5 ± 18.6	16	129.0 ± 11.2	−0.04	0.939
D-dimer (ng/mL)	8	114.7 ± 66.5	12	149.9 ± 54.0	0.009	0.209
Protein C (µg/mL)	9	3.3 ± 0.6	16	3.4 ± 0.7	0.031	0.727
sCD62P (ng/10^6 platelets)	8	0.2 ± 0.0	16	0.1 ± 0.0	−0.253	0.194

[a]Pearson's correlation coefficient between age and biomarker for both male and female macaques.
[b]*P* value between male and female macaques calculated by using 2-tailed Student *t* test with equal variance.

sCD62P is commonly used as a soluble marker of in vivo platelet activation.[2,6,8,16]

Only a few commercially available coagulation assays have sufficient cross-reactivity for use with NHP specimens. Some reference ranges for coagulation parameters in rhesus macaques, including those for D-dimer and antithrombin III, have been reported previously[9,27]. Regarding antithrombin III, the range established in the current study (124.2 to 133.4 µg/mL) is somewhat higher than the previously reported range (72 to 100 µg/mL).[9] This finding may be due to increased sensitivity or interspecies cross-reactivity of our assay but does not suggest a biologically significant discrepancy between our study and previous ones, given the broad range of normal antithrombin III values in humans. Likewise, the range for D-dimer that we established in the current study (110.3 to 161.3 ng/mL) is somewhat lower than the previously reported normal range (220 to 540 ng/mL).[27] To our knowledge, the current study marks the first time that sCD62P and protein C concentration ranges have been reported for rhesus macaques. The ranges of sCD62P and protein C (0.12 to 0.14 ng/10^6 platelets and 3.18 to 3.62 µg/mL, respectively) in rhesus macaques are similar to reported human reference ranges (0.06 to 0.16 ng/10^6 platelets and approximately 3.6 µg/mL, respectively).[10,28]

The variation in the reported ranges in our study may be due to inherent differences in the cross-reactivity, specificity, and sensitivity of the assays used. In addition, most of the assays described for rhesus studies have been optimized for human use. The methods developed here have been tested and optimized specifically for use with rhesus macaque plasma samples. We surmise that our assays are likely to be useful for other NHP samples, because we have achieved similar measurements in preliminary evaluations of samples from cynomolgus macaques

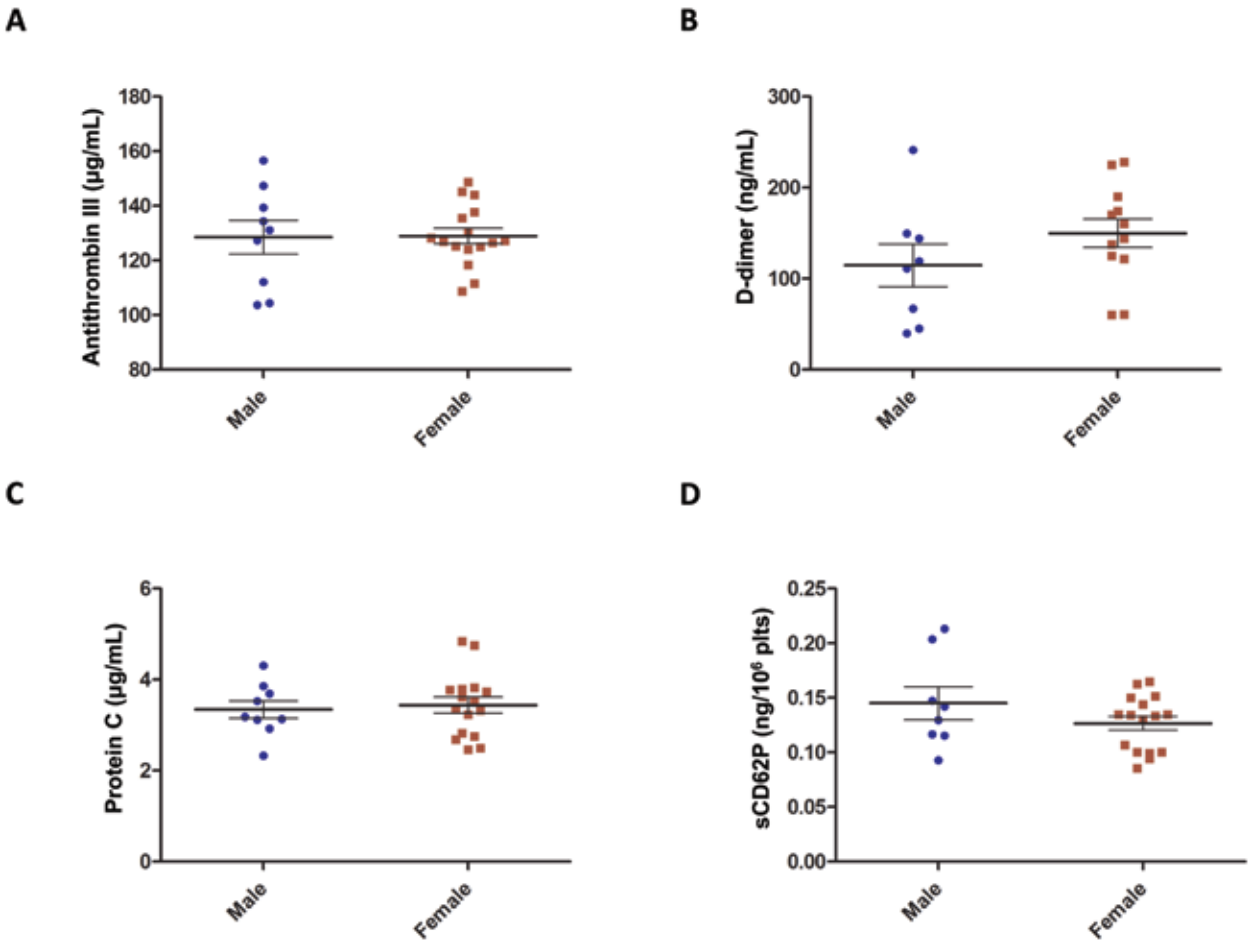

Figure 4. Coagulation biomarker measurements in male and female rhesus macaques (middle horizontal bar, mean; error bars, +/-1 SD). (A) Antithrombin III. (B) D-dimer. (C) Protein C. (D) sCD62P.

Table 5. Plasma levels of soluble P-selectin in male rhesus macaques

Macaque no.	sCD62P (ng/mL)	Platelet count (x10^6/mL)	sCD62P (ng/10^6 platelets)
1	77.6	381	0.20
2	28.4	306	0.09
3	58.4	274	0.21
4	43.6	378	0.12
5	51.2	395	0.13
6	40.8	350	0.12
7	57.2	388	0.15
8	83.2	586	0.14
Mean ± 1 SD	55.1 ± 18.4	382 ± 93	0.15 ± 0.04

Table 6. Plasma levels of soluble P-selectin in female rhesus macaques

Macaque no.	sCD62P (ng/mL)	Platelet count (x10^6/mL)	sCD62P (ng/10^6 platelets)
1	43.6	343	0.13
2	53.6	359	0.15
3	48.4	466	0.10
4	38.8	393	0.10
5	59.6	414	0.14
6	55.2	364	0.15
7	44.8	524	0.09
8	46.0	345	0.13
9	51.2	315	0.16
10	48.8	456	0.11
11	40.8	433	0.09
12	46.8	347	0.13
13	46.4	345	0.13
14	52.4	389	0.13
15	48.0	291	0.16
16	56.4	568	0.01
Mean ± 1 SD	48.8 ± 5.7	397 ± 76	0.13 ± 0.03

(*Macaca fascicularis*). These assays should be optimized for use in other species. Furthermore, when developing new assays, the intra- and interassay CV are ideally maintained at less than 10% and less than 20%, respectively, to ensure reliable results.[3] In our assays, there was a 12.2% intraassay variability for D-dimer and a 25.6% interassay variability for protein C (Table 2); the parameters for the other assays reported fell within the desired ranges. Human error is the most likely cause for this increased variability, because the plasma dilution required for the assays may be as high as 1:10,000, thereby increasing the risk of error.[32]

Our study has several strengths, including the presence of both male and female macaques across a range of ages, and the choice of assay platform used. The many advantages to using a bead-based ELISA format for these assays include high sensitivity, quantitative readouts, and the potential for multiplexing,

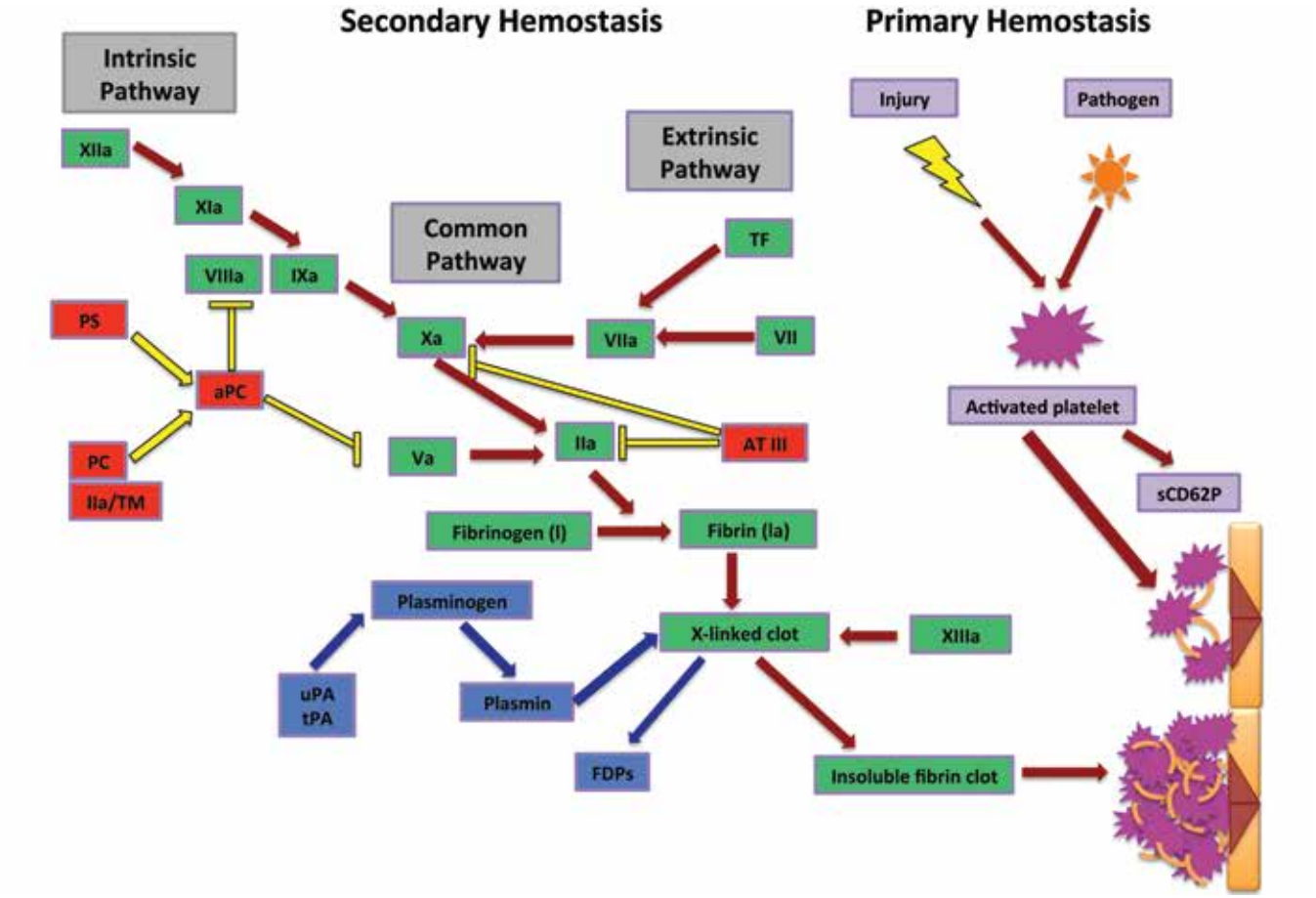

Figure 5. Schematic diagram representing the 4 main arms of coagulation: platelets (purple), coagulation (green), anticoagulation (red), and fibrinolysis (blue). Primary hemostasis results in the initiation of platelet aggregation at the site of stimulation and sCD62P release. Secondary hemostasis has 2 main pathways, intrinsic and extrinsic, which intersect at the common pathway, beginning at factor X. Antithrombin III and protein C are major anticoagulant proteins; antithrombin II inhibits factors IIa and Xa, whereas protein C inhibits Va and VIIa. Fibrinolysis can occur via plasmin cleaving crosslinked fibrinogen, resulting in fibrin degradation products, including D-dimer. PS, protein S; protein C, protein C; TM, thrombomodulin; uPA, urokinase plasminogen activator; tPA, tissue plasminogen activator; FDP, fibrin degradation products; TF; tissue factor; ATIII, antithrombin III; sCD62P, soluble P-selectin; and I through XIII are coagulation factors, with 'a' indicating activation.

which accommodates the measurement of multiple coagulation biomarkers from just microliters of plasma. Some limitations of our approach include the lack of species-specific reagents for NHP. The current assays were developed by using antibodies developed against human recombinant proteins. In light of the sequence homology of the *Homo sapiens* and *Macaca mulatta* proteins, we presume that there is sufficient species cross-reactivity with these reagents to provide clinically useful readings. This conclusion is supported by the fact that the ranges of values we obtained in this study are similar to the reference ranges reported for humans. Another limitation to the current study was the small sample size of normal healthy adult rhesus macaques included.[15] Although reference ranges can be calculated from as few as 20 to 40 subjects, ideally a pool of more than 120 subjects is used.[15] To establish official reference ranges for rhesus macaques by using the developed assays, a larger sample size with geographic variability should be analyzed to confirm the normality of sample distribution.

The development and validation of assays specific for rhesus macaques are important in the clinical evaluation of the hemostatic system. We have developed and validated 4 assays for the evaluation of biomarkers representing the coagulation and anticoagulation systems as well as platelet activation. In addition, we report—for the first time—normal ranges for protein C and sCD62P in this healthy population of rhesus macaques. Future studies will be focused on expanding the coagulation biomarker panel, increasing the speed and sensitivity of these assays, and reducing the total volume of sample required.

Acknowledgments

Financial support was provided by grants T32-0D019078-27 (to JGF), P30-ES002109 (to JGF), and P50-GM021700 (to RGT).

References

1. **Amin HM, Ahmad S, Walenga JM, Hoppensteadt DA, Leitz H, Fareed J**. 2000. Soluble P-selectin in human plasma: effect of anticoagulant matrix and its levels in patients with cardiovascular disorders. Clin Appl Thromb Hemost **6:**71–76.
2. **Andre P, Hartwell D, Hrachovinova I, Saffaripour S, Wagner DD.** 2000. Procoagulant state resulting from high levels of soluble P-selectin in blood. Proc Natl Acad Sci USA **97:**13835–13840.
3. **Angeloni S, Cordes R, Dunbar S, Garcia C, Gibson G, Martin C, Stone V.** 2013. Optimization of immunoassays, p 29–37. In: xMAP cookbook, 1st ed. Austin (TX): Luminex Corporation.
4. **Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M, Martin MJ, Natale DA, O'Donovan C, Redaschi N, Yeh LS.** 2004. UniProt: the Universal Protein knowledgebase. Nucleic Acids Res **32:** D115–D119.

5. **Ay C, Vormittag R, Dunkler D, Simanek R, Chiriac AL, Drach J, Quehenberger P, Wagner O, Zielinski C, Pabinger I.** 2009. D-dimer and prothrombin fragment 1 + 2 predict venous thromboembolism in patients with cancer: results from the Vienna Cancer and Thrombosis Study. J Clin Oncol **27:**4124–4129.
6. **Berman CL, Yeo EL, Wencel-Drake JD, Furie BC, Ginsberg MH, Furie B.** 1986. A platelet α-granule membrane protein that is associated with the plasma membrane after activation. Characterization and subcellular localization of platelet activation-dependent granule-external membrane protein. J Clin Invest **78:**130–137.
7. **Bernacky BJ, Gibson SV, Keeling ME.** 2002. Nonhuman primates, p 698–705. In: Fox JG, Anderson LC, Loew FM, Quimby FW editors. Laboratory Animal Medicine, 2nd ed. San Diego (CA): Elsevier.
8. **Bonfanti R, Furie BC, Furie B, Wagner DD.** 1989. PADGEM (GMP140) is a component of Weibel–Palade bodies of human endothelial cells. Blood **73:**1109–1112.
9. **Chen Y, Qin S, Ding Y, Wei L, Zhang J, Li H, Bu H, Lu Y, Cheng J.** 2009. Reference values of clinical chemistry and hematology parameters in rhesus monkeys (*Macaca mulatta*). Xenotransplantation **16:**496–501.
10. **Choudhury A, Chung I, Blann AD, Lip GY.** 2007. Platelet surface CD62P and CD63, mean platelet volume, and soluble/platelet P-selectin as indexes of platelet function in atrial fibrillation: a comparison of 'healthy control subjects' and 'disease control subjects' in sinus rhythm. J Am Coll Cardiol **49:**1957–1964.
11. **Cotroneo TM, Colby LA, Bergin IL.** 2011. Hemophagocytic syndrome in a pancytopenic simian retrovirus-infected male rhesus macaque (*Macaca mulatta*). Vet Pathol **48:**1138–1143.
12. **Eichinger BK.** 2000. Coagulopathic complications of cancer. In: Bast RC, Kufe DW, Pollock RE, Weichselbaum RR, Holland JF, Frei E editors. Holland–Frei cancer medicine, 5th ed. Hamilton (Canada): BC Decker.
13. **Esmon CT, Vigano-D'Angelo S, D'Angelo A, Comp protein C.** 1987. Anticoagulation proteins C and S. Adv Exp Med Biol **214:**47–54.
14. **Foley JE, Lerche NW, Dumler JS, Madigan JE.** 1999. A simian model of human granulocytic ehrlichiosis. Am J Trop Med Hyg **60:**987–993.
15. **Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J**. 2012. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol **41:**441–453.
16. **Furie B, Furie BC, Flaumenhaft R.** 2001. A journey with platelet P-selectin: the molecular basis of granule secretion, signalling, and cell adhesion. Thromb Haemost **86:**214–221.
17. **Hanna DL, White RH, Wun T.** 2013. Biomolecular markers of cancer-associated thromboembolism. Crit Rev Oncol Hematol **88:**19–29.
18. **Hubbard WJ, Hutchings A, Eckhoff D, Contreras J, Wilkins M, Thomas FT, Clements M, Thomas JM**. 2003. Particle-induced circulatory disturbances in transplanted rhesus macaque kidneys. Transp Int **16:**589–594.
19. **Kirk G, McLaren M, Belch JF.** 1997. Soluble P-selectin assay: importance of correct anticoagulant choice. Platelets **8:**159–162.
20. **McDermott MM, Greenland P, Guralnik JM, Ferrucci L, Green D, Liu K, Criqui MH, Schneider JR, Chan C, Ridker P, Pearce WH, Martin G, Clark E, Taylor L.** 2004. Inflammatory markers, D-dimer, prothrombotic factors, and physical activity levels in patients with peripheral arterial disease. Vasc Med **9:**107–115.
21. **Meyers K, Wardrop JJ.** 1991. Platelets and coagulation, p 87–136. In: Cotter SM, editor. Comparative transfusion medicine. San Diego (CA): Academic Press.
22. **Michelson AD, Barnard MR, Hechtman HB, MacGregor H, Connolly RJ, Loscalzo J, Valeri CR.** 1996. In vivo tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. Proc Natl Acad Sci USA **93:**11877–11882.
23. **Moreno A, Garcia A, Cabrera-Mora M, Strobert E, Galinski MR.** 2007. Disseminated intravascular coagulation complicated by peripheral gangrene in a rhesus macaque (*Macaca mulatta*) experimentally infected with *Plasmodium coatneyi.* Am J Trop Med Hyg **76:**648–654.
24. **Mosnier LO, Zlokovic BV, Griffin JH.** 2007. The cytoprotective protein C pathway. Blood **109:**3161–3172.
25. **Nazli C, Kinay O, Tunc B, Ergene O, Gedikli O, Ayata A, Dogan A, Altinbas A, Ergene U, Ozaydin M, Yavuz T, Kahraman H.** 2003. Diagnostic value of D-dimer and antithrombin III levels in predicting prosthetic heart valve thrombosis. Tex Heart Inst J **30:**268–279.
26. **Onlamoon N, Noisakran S, Hsiao HM, Duncan A, Villinger F, Ansari AA, Perng GC.** 2010. Dengue virus-induced hemorrhage in a nonhuman primate model. Blood **115:**1823–1834.
27. **Pandrea I, Cornell E, Wilson C, Ribeiro RM, Ma D, Kristoff J, Xu C, Haret-Richter GS, Trichel A, Apetrei C, Landay A, Tracy R.** 2012. Coagulation biomarkers predict disease progression in SIV-infected nonhuman primates. Blood **120:**1357–1366.
28. **Panwar R, Venkatesh B, Kruger P, Bird R, Gill D, Nunnink L, Dimeski G.** 2009. Plasma protein C levels in immunocompromised septic patients are significantly lower than immunocompetent septic patients: a prospective cohort study. J Hematol Oncol **2:**43.
29. **Perry DJ.** 1994. Antithrombin and its inherited deficiencies. Blood Rev **8:**37–55.
30. **Safronetz D, Prescott J, Feldmann F, Haddock E, Rosenke R, Okumura A, Brining D, Dahlstrom E, Porcella SF, Ebihara H, Scott DP, Hjelle B, Feldmann H.** 2014. Pathophysiology of hantavirus pulmonary syndrome in rhesus macaques. Proc Natl Acad Sci USA **111:**7114–7119.
31. **Sammak RL, Rejmanek DD, Roth TM, Christe KL, Chomel BB, Foley JE.** 2013. Investigation of tularemia outbreak after natural infection of outdoor-housed rhesus macaques (*Macaca mulatta*) with *Francisella tularensis.* Comp Med **63:**183–190.
32. **Schutheiss OC, Stanton SJ.** 2009. Assessment of salivary hormones. In: Harmon-Jones E, Beer JS editors. Methods of social neuroscience. New York (NY): Guilford Press.
33. **Tomlinson L, Boone LI, Ramaiah L, Penraat KA, von Beust BR, Ameri M, Poitout-Belissent FM, Weingand K, Workman HC, Aulbach AD, Meyer DJ, Brown DE, MacNeill AL, Bolliger AP, Bounous DI.** 2013. Best practices for veterinary toxicologic clinical pathology, with emphasis on the pharmaceutical and biotechnology industries. Vet Clin Pathol **42:**252–269.
34. **Twenhafel NA, Mattix ME, Johnson JC, Robinson CG, Pratt WD, Cashman KA, Wahl-Jensen V, Terry C, Olinger GG, Hensley LE, Honko AN.** 2013. Pathology of experimental aerosol Zaire ebolavirus infection in rhesus macaques. Vet Pathol **50:** 514–529.
35. **VandeBerg JL.** 1995. Genetics of nonhuman primates, p 129–146. In: Taylor B, Abee CR, Henrickson R editors. Nonhuman primates in biomedical research: biology and management. San Diego (CA): Academic Press.
36. **Weisel JW, Dempfle CE.** 2013. Fibrogen structure and function, p 254–271. In: Marder VJ, Aird WC, Bennett JS, Schulman S, White II GC, editors. Hemostasis and thrombosis, 6th ed. Philadelphia (PA): Lippincott Williams and Wilkins.