Hemostasis refers to the physiologic processes that maintain blood flow within the vascular space. Normal hemostasis involves plasma factors and cells (i.e., platelets, white and red blood cells, endothelial cells) at the site of vessel injury, and disease conditions affecting any of these components may cause signs of a bleeding diathesis. The most common bleeding disorders arise from a lack of functional platelets or coagulation factors. Therefore, initial diagnostic evaluation should include platelet count and coagulation screening tests, with interpretation and additional testing based on the context of each patient’s clinical presentation.

**DIAGNOSING COAGULATION DISORDERS**

**Coagulation Screening Tests**

Coagulation screening tests are conducted to detect factor deficiencies in patients with clinical signs of hemorrhage or disease conditions known to impair hemostasis. These tests are functional assays that measure the time for in vitro fibrin clot formation in samples of blood or plasma. Although in vivo fibrin generation results from complex interactions between procoagulant and anticoagulant factors, coagulation screening tests are configured with specific reagents to sequentially activate distinct series of coagulation factors (i.e., pathways) within the coagulation cascade. The prothrombin time (PT) is initiated by the addition of tissue factor and calcium to the test sample and is a measure of the extrinsic and common pathways (Figure 1). Patients deficient in one or more factors in this pathway demonstrate prolonged clotting time in the PT. Clot formation in the activated partial thromboplastin time (aPTT) is initiated by a reagent containing phospholipid and negatively charged contact particles followed by the addition of calcium to the reaction mixture. This test is sensitive to factor deficiencies in the intrinsic and common pathways (Figure 2). The activated clotting time (ACT) is a simplified test of this pathway; however, the ACT is influenced by nonspecific factors such as platelet count, platelet dysfunction, hematocrit, and plasma protease activity. The thrombin clotting time (TCT) and fibrinogen assays are con-
ducted by adding an excess of thrombin (factor IIa) to the test plasma. These tests measure the conversion of fibrinogen to fibrin and are sensitive only to deficiency, dysfunction, or inhibition of fibrinogen. Therefore, the pattern of abnormalities in coagulation screening tests depends on which factor(s) the patient lacks (Figure 3).

**Sample Collection for Coagulation Testing**

Special attention to blood collection technique is required to obtain quality samples for coagulation screening tests and specific factor analyses. Activation of platelets and coagulation factors during sample collection affects all subsequent tests and may result in spurious diagnoses of coagulopathy due to ex vivo factor depletion. Sample activation can be prevented by drawing blood directly into a premeasured volume of sodium citrate anticoagulant to obtain a final ratio of one part citrate to nine parts blood. Either 3.2% or 3.8% citrate plasma is acceptable for coagulation testing. However, heparin and EDTA plasma, serum, and samples drawn in clot activator tubes are all invalid for screening tests or factor analyses. To minimize factor consumption or contact activation, anticoagulated whole blood samples should be analyzed using a point-of-care device or centrifuged to separate plasma from cells as soon as possible after collection. Citrate plasma stored frozen retains factor activity, and shipment on cold packs for next-day delivery is acceptable for transport to testing laboratories. Optimal reaction conditions vary between species, and it is preferable to send samples for feline coagulation testing to laboratories with established feline (rather than human) reference intervals and controls.

**Factor Deficiencies in Acquired and Hereditary Coagulation Disorders**

The most common acquired coagulation factor deficiencies in dogs and cats are caused by liver disease, anticoagulant rodenticide toxicity, disseminated intravascular coagulation, envenomation, and treatment with warfarin sodium or heparin anticoagulants. The pathogenesis of factor deficiency in these disease syndromes includes failure of hepatic protein synthesis, vitamin K deficiency, systemic thrombin consumption, and fibrinogen and factor inhibition or depletion. These conditions affect multiple clotting factors within the intrinsic, extrinsic, and common pathways and typically cause prolongation of clotting time in more than a single screening test. For example, the pattern of a long aPTT and PT, with a normal TCT and fibrinogen, is characteristic of coagulopathy due to combined functional deficiency of the vitamin K–dependent factors (i.e., II, VII, IX, and X) (Figure 3). Marked prolongation of all screening tests with fibrinogen deficiency develops in patients with severe hepatic failure because most factors and fibrinogen are synthesized exclusively in the liver (Figure 3).

In contrast to combined factor deficiencies, the lack of a single clotting factor is almost always caused by a hereditary defect with mutation in the corresponding coagulation factor gene. Hereditary factor deficiencies may cause prolongation of a single coagulation screening test if that factor activity is restricted to the intrinsic or extrinsic pathway. Factor VII deficiency causes specific prolongation of PT (Figure 1), whereas isolated prolongation of aPTT (and ACT) is characteristic of factor VIII, IX, and XI and contact factor deficiencies (Figure 2). Coagulation factors VIII and IX are...
located on the X chromosome; all other clotting factor genes are located on autosomes. In humans, factor VIII deficiency (hemophilia A) is by far the most common hereditary factor deficiency, with an estimated incidence of one per 10,000 male births. Factor IX deficiency (hemophilia B) is second in frequency, with an estimated incidence of one per 40,000 male births. The high incidence of these defects is attributed to their X-linked recessive expression pattern and apparently high spontaneous mutation rates in factor VIII and IX genes.

As in humans, hemophilia A and B are the most common hereditary clotting factor deficiencies in dogs, with the same observed case ratio of approximately 4:1. In contrast, factor XII deficiency is considered the most common hereditary factor deficiency in cats. A high frequency of factor XII deficiency is unexpected based on comparative studies because the estimated incidence in humans is only one per 1 million births.

**COAGULATION FACTOR XII**

**Biologic Role**

Although factor XII deficiency causes marked prolongation of clotting time in the intrinsic system screening tests, its deficiency does not cause a clinical bleeding diathesis. Differences between physiologic fibrin generation and the in vitro reactions of the intrinsic pathway explain this apparent paradox. Factor XII is a proenzyme that autoactivates (to form the active serine protease factor XIIa) on contact with negatively charged surfaces. The reagents or collection tubes used for the aPTT and ACT assays provide artificial surfaces that support factor XIIa formation. Factor XIIa then initiates activation of the other components of the con-
Contact system: high molecular weight kininogen and prekallikrein. Factor XII also serves as a substrate of prekallikrein in a positive feedback cycle that amplifies this process of contact activation. The contact factor system activates factor XI, which in turn activates the intrinsic pathway, leading to thrombin generation and formation of a fibrin clot in the reaction mixture (Figure 2). In contrast to these in vitro reactions, physiologic thrombin generation is initiated by tissue factor–mediated activation of factor VIIa and then sustained and amplified by the factor IXa–factor VIIIa complex assembled on platelet membrane surfaces. Therefore, in vivo fibrin formation does not require the action of factor XII or the contact group factors. Comparative studies of intrinsic coagulation also reveal that birds and cetaceans lack detectable factor XII activity, further confirming that in vivo hemostasis is not factor XII dependent.

Factor XII and the contact group play a physiologic role in promoting complement activation, inflammation, fibrinolysis, and changes in vascular permeability. Contact factors assemble on the membrane surfaces of activated inflammatory cells and endothelium. Their interaction with membrane surface receptors results in the release of vasoactive and proinflammatory mediators such as kallikrein, bradykinin, and other kininogens. Once formed, kallikrein and factor XIIa activate the profibrinolytic proteins plasminogen and urokinase, thereby initiating and sustaining fibrin degradation. Although factor XII participates in these processes, its deficiency does not impair the in vivo assembly or activity of the contact group. It appears that the cell surface reactions of the contact group do not require the presence of plasma factor XII.

**Literature Review of Factor XII Deficiency**

Human factor XII deficiency was first described in 1955 and named Hageman trait for the index patient. This patient did not experience abnormal bleeding, and subsequent case series confirmed that factor XII deficiency does not cause a clinical bleeding diathesis in humans. Although early case reports suggested that factor XII deficiency might induce a hypercoagulable state, recent reviews of Hageman trait do not support causality for a thrombotic syndrome. Many suspect cases of mild to moderate factor XII deficiency were
found to be spurious and were actually caused by the antiphospholipid antibody syndrome, a confirmed risk factor for thrombosis. The antiphospholipid antibody syndrome causes prolonged in vitro clotting time but can be differentiated from true factor XII deficiency using quantitative protein assays and functional tests based on colorimetric, rather than fibrin clot, endpoints.

Hageman trait in humans is most often inherited in an autosomal recessive pattern. In this form, heterozygous carriers have intermediate factor activities ranging from 20% to 50% of normal, whereas homozygotes have marked reduction (i.e., <1%) in factor XII activity. The human disease phenotype is further characterized based on the presence of immunologically detectable factor XII protein, referred to as cross-reacting material. The human factor XII gene has been cloned, sequenced, and localized to chromosome 5. Molecular analyses of the factor XII gene from many affected patients reveal that Hageman trait is mutationally heterogeneous. Unrelated families have different mutations located at different functional sites in the factor XII gene. Most of the reported mutations are single-base changes that cause frameshifts in translation or alterations in gene splicing that ultimately abolish production of an intact factor XII gene product. Compilation of the various factor XII mutations has provided insight into gene regions and specific nucleotides required for secretion and activity of factor XII and related serine protease factors.

An early report of feline factor XII deficiency was published in 1959, followed almost 20 years later by detailed laboratory characterization of the intrinsic pathway of a factor XII–deficient female Domestic Shorthair cat. Subsequent studies of this cat’s progeny revealed that factor XII deficiency was transmitted as an autosomal recessive trait. Heterozygous cats had factor XII activities that were approximately 50% of normal, and homozygotes had values of less than 2%. The founder cat and her factor XII–deficient offspring had no evidence of a bleeding diathesis. Primary hemostasis was also studied in homozygous factor XII–deficient cats by assessment of oral (buccal) mucosa bleeding time. The in vivo bleeding times of factor XII–deficient cats were not significantly different from those of control cats, which is indicative of normal platelet plug formation in the absence of factor XII. Congenital (presumed hereditary) factor XII deficiency has been reported in client-owned Domestic Shorthair and Siamese-type cats from the northwestern and southern United States as well as the United Kingdom. In each of these clinical reports, observed signs of abnormal hemostasis were attributed to underlying disorders, such as thrombocytopenia, platelet dysfunction, and hemophilia, rather than a lack of factor XII.

Feline Factor XII Deficiency in the United States

The clinical characteristics of feline factor XII deficiency were summarized from a retrospective review of the Comparative Coagulation Laboratory’s case records for a 6-year interval (December 1998 to December 2004). Citrate plasma samples were submitted from referral veterinarians and assayed on the day of receipt using previously reported reagents and methods.

Figure 4. Collection of citrate plasma for coagulation testing. A procedure is outlined for collecting and processing citrate plasma for coagulation screening tests and coagulation factor analyses. Coagulation test services are available through the Comparative Coagulation Laboratory (www.diaglab.vet.cornell.edu/coag/submission).

Conduct in-house assay or store plasma frozen (ship on cold packs for next-day delivery to a referral laboratory)

Aspirate plasma and transfer it to a plastic tube

Centrifuge whole blood for 10–15 min

Draw exactly 2.7 ml of blood into the syringe

• Via clean venipuncture
• Via an indwelling catheter (after withdrawing and discarding a 3- to 4-ml purge sample)

Place exactly 0.3 ml citrate in a 3-ml syringe

Aspirate plasma and transfer it to a plastic tube

Centrifuge whole blood for 10–15 min

Draw exactly 2.7 ml of blood into the syringe

• Via clean venipuncture
• Via an indwelling catheter (after withdrawing and discarding a 3- to 4-ml purge sample)

Conduct in-house assay or store plasma frozen (ship on cold packs for next-day delivery to a referral laboratory)
Table 1. Signalment and Geographic Distribution of 51 Cats with Hereditary Factor Deficiencies

<table>
<thead>
<tr>
<th>Defect</th>
<th>Number of Cats</th>
<th>Gender</th>
<th>Median Age (range; yr)</th>
<th>Affected Breeds</th>
<th>Geographic Locationa (Number of cats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XII deficiency</td>
<td>30</td>
<td>18 Females 12 Males</td>
<td>3 (0.5–16)</td>
<td>20 DSH</td>
<td>Northeast (4)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>2 DLH</td>
<td>South (4)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>6 Siamese</td>
<td>Southeast (20)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1 Himalayan</td>
<td>Midwest (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 Breed unknown</td>
<td>West (2)</td>
</tr>
<tr>
<td>Hemophilia A (factor VIII deficiency)</td>
<td>13</td>
<td>13 Males</td>
<td>0.75 (0.3–10)</td>
<td>11 DSH</td>
<td>Northeast (6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 Birman</td>
<td>South (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 Breed unknown</td>
<td>Midwest (3)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>West (2)</td>
</tr>
<tr>
<td>Hemophilia B (factor IX deficiency)</td>
<td>8</td>
<td>8 Males</td>
<td>0.5 (0.5–3)</td>
<td>5 DSH</td>
<td>Northeast (4)</td>
</tr>
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<td></td>
<td></td>
<td>1 DLH</td>
<td>South (2)</td>
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<td></td>
<td>1 Persian</td>
<td>Midwest (1)</td>
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<td></td>
<td></td>
<td></td>
<td>1 Himalayan</td>
<td>West (1)</td>
</tr>
</tbody>
</table>

aRegions of the United States as defined by the United States Census Bureau (www.census.gov/geo/www/us_regdiv.pdf).

DLH = Domestic Longhair cat; DSH = Domestic Shorthair cat

Feline cases were selected for initial review based on low factor XII activity (FXII:C ≤50%). Cases were then excluded from subsequent analyses if factor XII deficiency was accompanied by concomitant deficiencies of other factors or fibrinogen. Factor XII deficiency in the excluded cases was considered to reflect an acquired disease process or sampling artifact rather than Hageman trait. Signalment, history, and results of coagulation testing were compiled from the remaining cats’ records. These parameters and the relative frequency of feline factor XII deficiency were compared with factor VIII deficiency (hemophilia A) and factor IX deficiency (hemophilia B) diagnosed during the review period.

A total of 1,422 feline samples were submitted for coagulation testing during the 6-year review period. Fifty-two cats (3.5% of cases) had hereditary intrinsic factor deficiencies, including 30 factor XII–deficient cats, 13 cats with hemophilia A, eight cats with hemophilia B, and one factor XI–deficient cat. The relative case ratios of factor XII deficiency to hemophilia A and B were approximately 2:1 and 4:1, respectively. The signalment and geographic location of the 51 Hageman trait and hemophilic cats are summarized in Table 1. All hemophilic cats were male, whereas 18 of 30 (60%) of the factor XII–deficient cats were female. Marked differences were also apparent in age at diagnosis. The median age for cats with hemophilia A or B was younger than 1 year, with only one cat older than 5 years of age at diagnosis. The factor XII–deficient cats’ median age was 3 years, and close to half of these cats (i.e., 13 of 30) were older than 5 years of age at diagnosis. Breed information was known for 49 of 51 cats (Table 1). Domestic Shorthair and Longhair breeds comprised close to 80% of cases and 84% of all feline submissions during the study period. Siamese cats were significantly overrepresented (i.e., 12% of cases) compared with only 4% of total feline submissions ($\chi^2 = 20.78$; 1 degree of freedom; $P < .001$). Factor XII–deficient cats also had an apparent geographic skew (Table 1), with 20 of 30 factor XII–defi-

**Factor XII–deficient cats do not require transfusion to replace factor XII before surgery or support hemostasis after injury.**
cient cats submitted from midwestern states compared with seven of 21 hemophilia cases. The excess number of factor XII–deficient cats from the Midwest was even more pronounced compared with the total feline caseload from the Midwest (i.e., only 8.5%; $x^2 = 134.31; 1$ degree of freedom; $P < .0001$).

All hemophilic cats had moderate to severe factor deficiencies, with values less than 5% of the feline standard plasma. The median factor VIII activity of cats with hemophilia A was 2.5% (range: <1% to 4.5%), and greater, whereas four cats having factor XII activities greater than 15% had aPTT values less than 60 seconds.

Case records included history or clinical signs for 41 of 51 factor-deficient cats (i.e., eight of 13 with hemophilia A, eight of eight with hemophilia B, and 25 of 30 with factor XII deficiency). At the time of diagnosis, all hemophilic cats had experienced one or more episodes of abnormal bleeding. The reported signs included subcutaneous and intramuscular hematoma formation, prolonged bleeding after castration or onychectomy, and gingival bleeding from tooth eruption sites. In contrast, factor XII deficiency was usually diagnosed in the course of preoperative screening after an initial finding of a long aPTT. The most common procedure prompting coagulation testing in factor XII–deficient cats was liver biopsy (12 cats), followed by endoscopy and gastrointestinal biopsy (four), ovariohysterectomy (three), and thyroidec- tomy (one). The indication for coagulation testing in the remaining factor XII–deficient cats included hema- tochezia (two cats), hematuria (one), glaucoma (one), and anemia (one). Nine of 15 factor XII–deficient cats had been spayed or neutered with no bleeding complica- tions before subsequently undergoing coagulation testing because of an acquired disease process.

**CONCLUSION**

Factor XII deficiency (Hageman trait) accounted for 2% of feline diagnoses at our referral coagulation laboratory and exceeded the combined number of cats with hemophilia A and B diagnosed during the 6-year review period.

Hereditary factor XII deficiency (Hageman trait) is a rare coagulation factor deficiency in most species but the most common factor deficiency in cats.
exotic breeds overlap with those of Domestic breeds. The apparent geographic bias of feline factor XII deficiency in the midwestern United States suggests introduction and subsequent propagation of a single mutation from a regional focus of Domestic breeds. Molecular analyses are ultimately needed to define the causative mutation(s) in factor XII–deficient cats from different breeds and regions. However, screening to eliminate mutations has limited clinical benefit because of lack of a bleeding diathesis associated with factor XII deficiency.

Most factor XII–deficient cats in this review had no signs or history of abnormal bleeding at the time of diagnosis. Factor XII deficiency was typically diagnosed in the course of a preoperative workup for elective surgery or acquired disease process after an initial finding of a long aPTT. In contrast, hemophilic cats were diagnosed at a relatively young age because of obvious signs of a bleeding diathesis. Hemophilia A and B are X-linked recessive traits, whereas the inheritance of factor XII deficiency is autosomal recessive. These differences in inheritance pattern explain the observed differences in sex distribution in the case review. Hemophilia A and B were diagnosed exclusively in male cats, and Hageman trait occurred in males and females.

Therefore, factor XII deficiency should be the suspected cause of a long aPTT in an adult cat lacking signs of a bleeding diathesis. Female gender, specific prolongation of aPTT beyond 60 seconds, and previous history of normal postoperative hemostasis lend further support to a diagnosis of hereditary Hageman trait. The definitive diagnosis can be accomplished through specific measurement of factor XII coagulant activity. Platelet count, coagulation screening tests, and fibrinogen analyses are indicated in the diagnostic evaluation of cats with clinical signs of abnormal bleeding, followed by more comprehensive factor analyses for complex cases.

REFERENCES

ARTICLE #4 CE TEST

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1. What is the reaction endpoint of all coagulation screening tests?
   a. color change in the reaction mixture
   b. formation of a fibrin clot
   c. strong agglutination reaction
   d. detection of a fluorescent probe
   e. cleavage of a chromogenic substrate

2. Prolongation of the aPTT with a normal PT and TCT is compatible with __________ deficiency.
   a. factor II
   b. factor VII
   c. factor VIII
   d. factor X
   e. fibrinogen

3. Prolongation of the PT with a normal aPTT and TCT is compatible with __________ deficiency.
   a. factor II
   b. factor VII
   c. factor VIII
   d. factor X
   e. fibrinogen

4. Which sample is valid for coagulation testing (screening tests and specific factor assays)?
   a. serum
   b. citrate plasma
   c. heparin plasma
   d. EDTA plasma
   e. whole blood with no anticoagulant

5. Deficiency of the contact group factors (i.e., factor XII, prekallikrein, and high molecular weight kininogen) causes prolongation of
   a. aPTT and ACT.
   b. TCT and fibrinogen.
   c. PT and TCT.
   d. PT and aPTT.
   e. ACT and TCT.

6. Factor XII does not participate in
   a. complement activation.
   b. fibrinolysis.

7. Which statement is correct?
   a. Factor XII deficiency is the most common hereditary clotting factor deficiency in humans.
   b. Hemophilia B is the most common hereditary clotting factor deficiency in humans.
   c. Hemophilia A is the most common hereditary clotting factor deficiency in humans.
   d. Factor XII deficiency has not been diagnosed in humans.
   e. Hemophilia A and factor XII deficiency occur with equal frequency in humans.

8. Which clinical or laboratory finding is suggestive of feline factor XII deficiency?
   a. petechiae
   b. long buccal bleeding time
   c. marked prolongation of TCT
   d. prolonged bleeding after surgery
   e. marked prolongation of aPTT

9. Which statement reflects the expected and observed frequency of feline factor XII deficiency?
   a. The ratio of male to female cases is nearly 20:1.
   b. Factor XII deficiency occurs exclusively in male cats.
   c. Factor XII deficiency occurs exclusively in female cats.
   d. The ratio of female to male cases is nearly 20:1.
   e. The ratio of male to female cases is nearly 1:1.

10. A diagnosis of factor XII deficiency has been confirmed in a healthy cat undergoing elective ovariohysterectomy. Which is appropriate preoperative management?
    a. No specific treatment is required.
    b. Perform a transfusion with type-matched feline fresh whole blood.
    c. Perform a transfusion with type-matched feline fresh-frozen plasma.
    d. Initiate low-dose heparin therapy.
    e. Initiate broad-spectrum antibiotic therapy.