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Abstract: Hemostasis, the coagulation cascade, and clot formation present a daunting list of factors, pathways, and interactions to equine clinicians. A basic knowledge of hemostasis is necessary to evaluate various disease processes in horses. Initial injury to the vascular endothelium results in local vasoconstriction and formation of a platelet plug. This initial response reduces blood loss from the damaged vessel but may be inadequate at maintaining hemostasis alone. Therefore, the intrinsic, extrinsic, and common coagulation pathways interact with one another to form thrombin and ultimately stabilize the platelet plug. Thrombin accomplishes this by converting soluble fibrinogen to insoluble fibrin, resulting in clot stabilization. Alternatively, fibrinolysis promotes resolution of clot formation. Because of multiple positive and negative feedback interactions as well as multiple circulating mediators and inhibitors, coagulation and fibrinolysis are finely controlled systems in healthy patients. However, perturbations of the coagulation cascade in disease states can result in severe complications or death.

Response to Initial Vascular Injury
The main function of the coagulation system is to control hemorrhage from the vascular endothelium when it has been damaged by trauma or inflammation. Conversely, anticoagulants and fibrinolytic mechanisms maintain patency and adequate blood flow. The regulation of these mechanisms is a tightly knit system of local and systemic feedback control pathways that maintains a delicate balance between pro- and anticoagulant processes.

Hemostasis is defined as the arrest of bleeding by vasoconstriction and coagulation and is an essential part of normal functioning of the cardiovascular system.
Hemostasis is usually initiated by vascular trauma but can be induced by inflammation or sepsis. Smooth muscle contraction marks the beginning of the hemostatic process and begins instantaneously after endothelial damage. Myogenic spasm of smooth muscle is followed by endothelial and platelet release of autacoid vasoconstrictive substances—endothelin and thromboxane, respectively. Further vasoconstriction caused by sensory nerve impulses results in a neurogenic contractile reflex of smooth muscle.

Damaged endothelium serves as a mediator and initiator of platelet adherence and formation of the platelet plug, known as primary hemostasis. Likewise, damaged endothelium activates the extrinsic and intrinsic coagulation enzyme cascades via tissue factor and collagen fiber exposure. The direct result of activating either or both coagulation cascades is conversion of prothrombin to thrombin; this subsequently results in the conversion of soluble fibrinogen to insoluble fibrin and stabilization of the clot (secondary hemostasis). Platelet adherence and activation combined with activation of the coagulation cascades result in a blood clot or thrombus formation to stop hemorrhage.

**Platelet Plug Formation and Primary Hemostasis**

Essential to progression of the clotting process is the development of a platelet plug via platelet adhesion, activation, recruitment, and aggregation (FIGURE 1). Platelets are circulating cytoplasmic fragments of megakaryocyte pseudopods. They contain actin–myosin molecules and thrombostenin for platelet contraction, an endoplasmic reticulum and Golgi apparatus for enzyme production and calcium storage, and a variety of enzymatic systems and secretory granules. Glycoproteins on the platelet surface and within platelet granules help mediate adherence to the vascular endothelium and serve as a means of communication and activation among platelets. After vascular injury, adherence is initiated largely by the binding of platelets to exposed subendothelial matrix via von Willebrand's factor (vWF). Fibrinogen binding to platelet glycoprotein complexes enhances the activation of additional platelets. Platelet aggregation ensues with further

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**TABLE 1 Coagulation Factor Synonyms**

<table>
<thead>
<tr>
<th>FACTOR</th>
<th>SYNONYM</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>III</td>
<td>Tissue factor, thromboplastin</td>
</tr>
<tr>
<td>IV</td>
<td>Calcium</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin, labile factor</td>
</tr>
<tr>
<td>VI</td>
<td>—</td>
</tr>
<tr>
<td>VII</td>
<td>Proconvertin, stable factor</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilic factor</td>
</tr>
<tr>
<td>IX</td>
<td>Christmas factor</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Prower factor</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromboplastin antecedent</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin-stabilizing factor, transglutaminase</td>
</tr>
</tbody>
</table>

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(C) Secondary hemostasis involves activation of the intrinsic and extrinsic pathways, culminating in the activation of prothrombin to thrombin via the common pathway of coagulation. (D) Clot stabilization occurs via fibrin fiber formation within the clot and subsequent clot stabilization by fibrin stabilizing factor (XIII).
Hemostasis

platelet activation, forming the platelet plug. Prothrombin attaches to platelet surface receptors as well, accumulating within the newly formed platelet plug. Platelet activation results in platelet swelling and the development of membrane surface pseudopods containing alpha granules and dense bodies. Alpha granules contain substances essential to hemostasis, including adhesive proteins for vWF, fibrinogen, plasminogen, fibronectin, and thrombospondin; plasma proteins IgG and albumin; cellular mitogens of platelet-derived growth factor; coagulation factors V and VIII; and protease inhibitors α2-macroglobulin and α2-antiplasmin.6 Dense bodies activate and recruit locally circulating platelets via release of ADP, ATP, serotonin, ionized calcium, histamine, epinephrine, and pyrophosphate.7 Fibronectin and thrombospondin help reinforce and stabilize aggregated platelets. Platelet-derived growth factor increases smooth muscle growth while controlling tissue repair. These factors facilitate and regulate the continual adherence, activation, and aggregation of platelets until a loose but reversible plug forms. Further platelet plug stabilization is attained through the activation of coagulation pathways and the generation of thrombin.

Endothelial Cell Reactions

Endothelial cells are responsible for initiating coagulation in response to local vascular injury or systemic inflammation.8 Intact endothelium maintains normal function and limits activation of coagulation by maintaining the smooth luminal surface of vessels. Endothelial production of the glycocalyx, a mucopolysaccharide, limits platelet adherence to the endothelium and platelet activation. Vessel trauma and resulting endothelial damage lead to exposure of subendothelial matrix, which is largely composed of collagen. The combined endothelial expression of vWF and tissue factor, in addition to subendothelial collagen exposure, activates the coagulation cascade and supports platelet adhesion and activation.5 vWF mediates the adhesion of platelets to exposed collagen fibrils while stabilizing factor VIII; the latter plays a role in secondary hemostasis. Tissue factor initiates secondary hemostasis via the extrinsic pathway. Collagen exposure simultaneously causes a change in configuration of the proteolytic enzyme factor XII, which signals the activation of the intrinsic pathway. In summary, the endothelium plays a key role in initiating, amplifying, and modulating the coagulation process in response to many conditions, including trauma, inflammation, sepsis, and endotoxemia.9,10

Secondary Hemostasis

Secondary hemostasis begins with the activation of the extrinsic and intrinsic pathways immediately after vessel damage. These pathways converge to form prothrombin activator, which catalyzes the formation of thrombin from prothrombin. Thrombin converts soluble fibrinogen to insoluble fibrin fibers that interact with platelets, blood cells, and plasma for final formation of a stable clot.

The Coagulation Cascade: the Extrinsic Pathway

Activation of the extrinsic pathway occurs via expression of tissue factor, a cellular lipoprotein, resulting from endothelial damage and subsequent subendothelial matrix exposure (FIGURE 1). Activated factor VII (VIIa), alone or in combination with calcium and tissue factor, forms a proteolytic complex composed of phospholipids and lipoproteins that enzymatically activates factor X.11 In addition to tissue factor, factor VII can be activated by factor IXa, Xa, XIIa, or XIIIa or thrombin and, when activated, catalyzes the activation of additional factor X.12 The common pathway proceeds with the activation of factor X to produce thrombin and, subsequently, fibrin. Thrombin's activation of factor VII is concentration dependent, with activation and inactivation occurring at lower and higher concentrations, respectively.13 Thrombin production is further regulated by the production of tissue factor pathway inhibi-
tor by the vascular endothelium when factor Xa is formed via the extrinsic pathway. Tissue factor pathway inhibitor inhibits the extrinsic pathway through binding of the tissue factor/calcium/factors VIIa and Xa complex. The small amount of thrombin that is produced rapidly catalyzes its own formation via activation of factor V and interacts with the intrinsic pathway by activating factor VIII. An interconnection between the extrinsic and intrinsic pathways exists through the activation of factor IX of the intrinsic pathway by a tissue factor–factor VIIa complex. Factor VII, which is produced by the liver, has the shortest half-life of the coagulation factors and is, therefore, significant during diagnostic evaluation of the extrinsic pathway and of liver function.

The Coagulation Cascade: the Intrinsic Pathway

Coagulation factors VIII, IX, XI, and XII are essential for proper function of the intrinsic pathway (FIGURE 1). Calcium ions, platelet phospholipids, and the proteins prekallikrein and high-molecular-weight kininogen (HMWK) are cofactors necessary for coagulation factor activation and positive feedback. Contact between factor XII and collagen fibers exposed by endothelial damage is the primary initiator of the intrinsic pathway. This stimulates a conformational change and activation of the proteolytic enzyme to factor XIIa. Conversion of prekallikrein to kallikrein by factor XIIa can also activate factor XII, and additional factor XIIa results in further conversion of prekallikrein to kallikrein. This positive feedback mechanism results in amplification of the intrinsic pathway. In the presence of HMWK, factor XIIa converts factor XI; and this process is accelerated by prekallikrein. Bradykinin is released from HMWK in this process, acting as a potent vasodilator, and counterbalances other vasoconstrictive substances released with vessel damage. The enzymatic action of factor Xa activates factor IX. Factor IX contains vitamin K–dependent γ-carboxyglutamate (Gla) and is calcium dependent (calcium binds and activates Gla). Vitamin K is a cofactor in the carboxylation of glutamate of coagulation proteins; it is essential for the conformational change and calcium chelation that occur with factor activation, specifically factors II (prothrombin), VII, IX, and X. Coumarin-type anticoagulants inhibit vitamin K–dependent epoxide reductase and carboxylation of coagulation factors, thus limiting factor synthesis in the liver and activation at the site of vascular injury. The combination of factor IXa, factor VIIIa, platelet phospholipid, tissue factor, and calcium ions completes the intrinsic pathway process through activation of factor X. The complex of calcium and factors VIIIa, IXa, and X is found on the surface of platelets and is known as the tenase complex. Exposure of platelet phospholipids allows the tenase complex to form. The entire complex is then regulated by activation of factor VIII by thrombin. Assembly of the tenase complex on a platelet phospholipid foundation in the presence of calcium allows clot formation to remain localized.

Factor VIII is considered a cofactor in the coagulation cascade and is activated by the presence of thrombin. Factor VIII works as a receptor for factors IXa and X. As described above, the conversion of factor VIII to VIIIa depends on the thrombin concentration, with a high concentration resulting in factor VIIIa cleavage and inactivation. This inherent mechanism serves as a local means of coagulation regulation.

Common Pathway

The intrinsic and extrinsic pathways converge at the common pathway, resulting in prothrombin activation, which occurs on platelet surfaces and requires formation of the prothrombinase complex. Components of the prothrombinase complex include phosphatidylinositol and phosphatidylserine (procoagulant platelet phospholipids), tissue phospholipids (from tissue factor), calcium ions, factors Va and Xa, and prothrombin. Within a few seconds, prothrombinase catalyzes the conversion of prothrombin to thrombin in the presence of calcium. Factor Va functions as a cofactor in prothrombinase complex formation and is bound to the surface of platelets. Factor X represents the actual protease responsible for the conversion of prothrombin to thrombin, which can be accelerated by factor Va and platelet phospholipid. The proteolytic action of thrombin to form factor Va catalyzes the formation of additional thrombin. Factor V is activated by a low thrombin concentration and is gradually inactivated as the thrombin concentration increases. This demonstrates a local feedback mechanism of coagulation.

Critical Point

Platelet adhesion and aggregation at the site of initial vascular injury form the platelet plug, which marks the beginning of primary hemostasis. This is essential for the coagulation process to proceed.
Clot Formation

The final phases of clot formation are characterized by the conversion of fibrinogen to fibrin by thrombin. Thrombin acts through proteolytic cleavage of fibrinogen to a fibrin monomer. The polymerization of fibrin monomers leads to fibrin fiber formation. Initially, weak fibrin bonds progress to form the long fibers of the clot reticulum. The conversion and activation of factor XIII (fibrin-stabilizing factor) by thrombin help develop covalent bonds between fibrin fibers to further strengthen the clot. Fibrin-stabilizing factor is supplied by platelets and circulating plasma globulins in the clot reticulum.

As the meshwork of fibrin fibers grows, circulating red blood cells, platelets, and plasma become trapped in damaged areas. This combination leads to the recruitment of platelets and coagulation factors necessary for clot expansion and aggregation, eventually stopping vessel hemorrhage. Clot retraction begins within 20 to 60 minutes after formation. Platelet release of procoagulants from alpha granules, as well as factor XIII, further stabilizes the bonding of fibrin fibers. Platelet contractile proteins thrombosthenin, actin, and myosin are stimulated to contract platelet spicules attached to adjacent fibrin fibers. Calcium ions from platelet stores and thrombin production facilitate further clot contraction. This process continues as the edges of the damaged vessel are pulled together in conjunction with smooth muscle contraction until hemorrhage ceases and hemostasis is achieved.

Thrombin

Thrombin plays a pivotal role in all aspects of hemostasis. Thrombin has multiple “avenues” of positive feedback to accelerate further thrombin production. The catalysis of factors V, VII, VIII, and XI drives the extrinsic and intrinsic coagulation pathways. Other functions of thrombin include platelet activation, aggregation and secretion, and smooth muscle contraction. The ability of thrombin to influence these reactions is concentration dependent, as described above, and serves as a mechanism of local feedback to control clot formation. As the formation of thrombin accelerates, so does the production of anticoagulant reactions necessary for clot dissolution. The binding of thrombin to thrombomodulin, tissue plasminogen activator (TPA), and activated protein C are examples of thrombin’s ability to initiate clot breakdown.

Clot Prevention

Endogenous anticoagulants maintain vessel patency by limiting intravascular coagulation caused by daily trauma to the vascular endothelium. Induction of the contact phase and tissue factor pathway of the intrinsic and extrinsic pathways, respectively, is limited by the endothelial production of the glycocalyx, which lines the endothelium to produce a smooth surface. Tissue factor pathway inhibitor serves as an important endogenous mechanism of controlling hemostasis by directly inhibiting the formation of the factor Xa–factor VIIIa complex.

Inhibition of thrombosis occurs via multiple mechanisms. Thrombin can bind thrombomodulin, resulting in activation of protein C and proteolysis of factors Va and Villa. In addition, antithrombin inhibits thrombin and factors Xila, Xla, Xa, and IXa. Antithrombin also binds heparin-like molecules (i.e., heparin sulfate) that are expressed on endothelial surfaces, which subsequently inhibits thrombin and factors Xa and IXa.
VIIa–tissue factor complex. The removal of thrombin can also control coagulation. Fibrin fibers themselves incorporate large amounts of thrombin within the clot reticulum. In addition, the binding of free thrombin by thrombomodulin activates plasma protein C, which inactivates factors Va and VIIIa via proteolysis, representing another aspect of negative feedback control of coagulation and down-regulation of thrombin (FIGURE 2). Protein C has many anticoagulant, profibrinolytic, and antiinflammatory actions and has been shown to decline during periods of sepsis.

Antithrombin inactivates the remaining thrombin in the immediate area of clot formation. Antithrombin is responsible for inactivation of factors IXa, Xa, XIa, and XIIa through thrombin binding. Endogenous or exogenous heparin binding to antithrombin results in a conformational change of the globulin and heightened affinity for thrombin. Further thrombin activity can also be limited by α2-macroglobulin, heparin cofactor II, and α1-antitrypsin.

**Fibrinolysis**

Hemostasis is followed by the return to normal vascular flow around, or adjacent to, the newly formed clot. Returning the damaged vessel to normal function requires the dissolution of the clot by fibrinolysis. Circulating plasminogen (profibrinolysin) becomes ensnared within the newly formed clot, binding to fibrin and fibrinogen. Trapped plasminogen is activated by endothelially synthesized TPA to form plasmin (FIGURE 3). Binding of TPA to fibrin is inhibited by plasminogen–activator inhibitors types 1 and 2. Urokinase, which is produced by epithelial cells lining excretory ducts, is also responsible for plasmin activation, to a lesser degree, and assists in dissolution of fibrin clots that may form in these ducts.

Plasmin causes proteolytic digestion of fibrin fibers, fibrinogen, prothrombin, and factors V, VIII, and XII. The digestion of fibrinogen and fibrin fibers results in the release of soluble fibrin degradation products (FDPs). The digestion by plasmin of insoluble cross-linked fibrin fibers during fibrinolysis causes the release of D-fragments or cross-link remnants, known as D-dimers. The action of plasmin is the final step in clot dissolution and can reopen previously blocked vessels. Renal and hepatic production of α2-antiplasmin inactivates free plasmin.

**Diagnostic Testing of Coagulation**

**Cytology**

Examination of a peripheral blood smear or automated quantification of platelet numbers is one avenue of assessing primary hemostasis (TABLE 2). Reported and established reference intervals for platelet concentration in an adult horse generally range from 100,000 to 300,000 platelets/µL. Six to 20 platelets per high-powered field (magnification: ×1000) is considered adequate when examining a peripheral blood smear, if clumping of platelets is not observed. Ten different fields must be examined and the

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**Critical Point**

Endogenous anticoagulants antithrombin and protein C limit intravascular coagulation from daily vascular trauma.
average number of platelets per field determined to adequately evaluate platelet concentration by this method. Platelet numbers must be interpreted carefully in horses because of the effects of splenic sequestration and collection methods. Splenic contraction can mildly increase circulating platelets. Also, the use of EDTA anticoagulant has been shown to falsely lower the platelet concentration. Therefore, the use of a sodium ion citrate blood tube (light blue–top tube) is recommended. Thrombocytopenia is classified as platelet concentration below 100,000/µL, and clinical bleeding has been associated with platelet concentrations below 30,000/µL. Platelet characteristics, granularity, size, shape, and clumping can also be evaluated on peripheral blood smear examination. However, granules in equine platelets are very faint compared with those of other species, making platelets more difficult to identify and granularity difficult to assess in horses. In addition, even if the platelet count is within normal limits, platelet function may not be normal, and platelet function testing is currently being evaluated in equine patients.

Template Bleeding Time
Template bleeding time evaluates endothelial interaction with circulating platelets. Template bleeding time is a cost-effective and easily performed evaluation of primary hemostasis, whereas other functional tests are specific to secondary hemostasis. Hair is clipped over the skin distolateral to the accessory carpal bone, and a template bleeding device is placed and discharged. Filter paper is used to absorb blood that is discharged from the incision 1 to 2 mm below the incision site. Timing starts when the incision is made and ends when the bleeding stops. The reported reference range for template bleeding time in healthy horses is 2 to 6 minutes. Thrombocytopenia and the functional platelet disorders von Willebrand's disease and Glanzmann's thrombasthenia can be screened using this test.

### Table 2 Diagnostic Testing of Hemostasis

<table>
<thead>
<tr>
<th>DIAGNOSTIC TEST</th>
<th>&lt; 24 HOURS</th>
<th>4–7 DAYS</th>
<th>10–14 DAYS</th>
<th>25–30 DAYS</th>
<th>ADULT</th>
<th>FACTORS EVALUATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (sec)</td>
<td>10.9 ± 0.6</td>
<td>9.6 ± 0.6</td>
<td>9.5 ± 0.4</td>
<td>9.4 ± 0.4</td>
<td>9.5 ± 0.3</td>
<td>Extrinsic and common</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>56.8 ± 6.3</td>
<td>39.8 ± 4.0</td>
<td>39.9 ± 4.8</td>
<td>40.8 ± 6.0</td>
<td>42.0 ± 8.9</td>
<td>Intrinsic and common</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>116.8 ± 39.1</td>
<td>196.8 ± 26.6</td>
<td>199.6 ± 50.0</td>
<td>221.1 ± 48.0</td>
<td>195 ± 54</td>
<td>Quantification of fibrinogen</td>
</tr>
<tr>
<td>Platelet count (10³/µL)</td>
<td>243 ± 170</td>
<td>181 ± 60</td>
<td>218 ± 57</td>
<td>245 ± 59</td>
<td>153 ± 49</td>
<td>Quantification of platelet numbers</td>
</tr>
<tr>
<td>FDPs (µg/mL +1)³</td>
<td>8.2 ± 2.7</td>
<td>5.6 ± 3.4</td>
<td>4.5 ± 3.1</td>
<td>3.5 ± 2.6</td>
<td>1.8 ± 0.6</td>
<td>Presence of fibrin from action of plasmin on fibrin or fibrinogen</td>
</tr>
<tr>
<td>Antithrombin activity (%)</td>
<td>107 ± 41</td>
<td>164 ± 35</td>
<td>170.9 ± 40.9</td>
<td>166.5 ± 40.6</td>
<td>202 ± 82</td>
<td>Evaluates antithrombin activity</td>
</tr>
<tr>
<td>D-dimers (ng/mL)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>677 ± 119</td>
<td>Specific evaluation for plasmin degradation of cross-linked fibrin</td>
</tr>
<tr>
<td>Template bleeding time (min)</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>Hemostatic plug formation⁶</td>
</tr>
<tr>
<td>Activated clotting time (min)</td>
<td>5.8 ± 1.3</td>
<td>5.8 ± 1.3</td>
<td>5.8 ± 1.3</td>
<td>5.8 ± 1.3</td>
<td>2.6 ± 0.5</td>
<td>Secondary hemostasis⁶</td>
</tr>
<tr>
<td>Thrombin time (sec)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>19.5 ± 5.5</td>
<td>Conversion time of fibrinogen to fibrin</td>
</tr>
</tbody>
</table>

*Listed in order of most used to least used.


N/A = reference intervals for a horse of this age are not available; PT = prothrombin time; aPTT = activated partial thromboplastin time; FDPs = fibrin degradation products.
Activated Clotting Time
Secondary hemostasis can be functionally assessed using the activated clotting time (ACT). A deficiency in factors V, VIII, IX, X, XI, and XII; prothrombin (factor II); and fibrinogen prolongs the ACT. The ACT is not affected by the blood concentration of factor VII or the platelet concentration and thus evaluates the intrinsic and common pathways. However, severe thrombocytopenia can result in a prolonged ACT because of decreased availability of phospholipid. The test is conducted by mixing whole blood directly with diatomaceous earth, incubating it at 98.6°F (37°C), tilting the sample back and forth, and recording the time to clot formation. The reference time for clot formation has been reported as 2 minutes and 38 seconds (± 29 seconds). Variations in the testing protocol, including syringe versus vacuum collection, incubation period and temperature, and frequency of monitoring for clot formation, account for the general low sensitivity of this test.

Partial Thromboplastin Time
Evaluation of the intrinsic and common pathways via the partial thromboplastin time (PTT) is similar to evaluation by the ACT, but the PTT has a higher sensitivity. Deficiency of factors V, VIII, IX, XI, and XII; prothrombin; and fibrinogen causes prolongation of the PTT. The test is conducted by adding citrated plasma to a glass tube with a phospholipid and calcium chloride substrate. The time until clot formation is then determined.

Activated Partial Thromboplastin Time
The activated partial thromboplastin time (aPTT) test is similar to that for the PTT, except that a contact activator (e.g., kaolin, silicates, ellagic acid) is added to the specimen when determining the aPTT. The aPTT is most commonly used to evaluate the intrinsic and common pathways. Prolongation of the PTT and aPTT in horses has been associated with hemophilia A (factor VIII deficiency) and plasma prekallikrein deficiency.

Prothrombin Time
The prothrombin time (PT) or one-stage PT test is an analysis of the extrinsic and common pathways, specifically their ability to convert fibrinogen to fibrin. Deficiencies of tissue factor (III); coagulation factors V, VII, and X; prothrombin; and fibrinogen result in prolongation of PT. The test is conducted by mixing citrated plasma with tissue factor and calcium. The time until fibrin fiber or clot formation is calculated automatically. Citrated whole blood or plasma can be used up to 3 days after collection with the concurrent submission of a control sample from a normal horse. Prolongation of PT occurs when the fibrinogen concentration decreases below 100 mg/dL, the coagulation factors listed above are reduced more than 50%, prothrombin decreases 30%, or vitamin K deficiency is present. Clinically relevant prolongation has been described as a 20% increase in clotting time compared with that of healthy horses.

Thrombin Time
The thrombin time test is an assessment of the terminal common pathway of secondary hemostasis and is a direct assessment of fibrinogen quantity and quality. FDPs interfere with fibrin polymerization and can prolong thrombin time. Hypofibrinogenemia also prolongs thrombin time. The test is conducted by the addition of thrombin to citrated plasma and is interpreted by the time interval for clot formation.

Proteins Induced by Vitamin K Absence or Antagonism
The proteins induced by vitamin K absence or antagonism (PIVKA) test is an evaluation of the proteins awaiting conversion to coagulation factors as well as proteins that inhibit coagulation. Factors II, VII, IX, and X and the anticoagulant proteins C and S depend on vitamin K carboxylation of glutamate from their protein precursor for normal hemostatic function. In the absence of vitamin K, continual synthesis of protein precursors causes a buildup within hepatocytes and a decline in functional coagulation factor production. The leakage of PIVKA into the peripheral circulation is quantified through the PIVKA test, which determines the clotting time with the addition of thromboplastin to the sample. Deficiencies in prothrombin and factors VII, IX, and X as well as anticoagulant poisoning can cause prolongation of the PIVKA test. The PIVKA test, in conjunction with the PT, has been widely used in diagnosing and confirming anticoagulant poisoning in dogs.
when results exceed 150 seconds. The use of the PIVKA test in horses has been limited to suspected cases of anticoagulant toxicity but could also be indicated in cases of feed toxicity (moldy sweet clover).

**Fibrinogen**
Evaluation of the fibrinolytic system through the fibrinogen concentration is less sensitive in horses than in other species. A decreased fibrinogen concentration can indicate disseminated intravascular coagulation (DIC), but given the capacity of the equine liver to produce large amounts of acute-phase proteins (fibrinogen) in response to inflammation, the fibrinogen level may be increased or remain unaltered despite profound inflammation. The fibrinogen concentration can be determined by a heat precipitation test that compares the plasma protein concentration before and after heating. A more sensitive mechanical method can be conducted by many coagulation analyzers.

**Fibrin Degradation Products**
FDPs are the result of the consumption of fibrinogen or cross-linked fibrin fibers by plasmin. Fibrin fragments are detected by mixing a blood sample with thrombin and a protease inhibitor. An increased FDP concentration suggests increased activities of fibrinolysis or fibrinogenolysis. Increased serum FDPs can occur when fibrin deposition exceeds normal clearance mechanisms, and they have been linked to DIC, severe inflammatory processes, hemorrhagic disorders, and postoperative states.

**D-dimers**
D-dimer production is the end result of fibrinolysis caused by plasmin activity. Specifically, it is the breaking of thrombin-induced fibrin fiber bonds with the release of D-fragments (dimers). D-dimer identification differs from testing for FDPs in that it is specific for dissolution, by plasmin, of fibrin generated from active thrombin production, whereas FDPs indicate general fibrinolysis and fibrinogenolysis. D-dimer testing in conjunction with FDP analysis has been used in assessing DIC in horses, but it is important to note that the D-dimer concentration alone is not specific for the process or diagnosis of DIC and, therefore, should not be overinterpreted.

**Antithrombin**
The hepatocyte-produced α-globulin antithrombin is considered one of the most important endogenous inhibitors of coagulation. The function of antithrombin is the neutralization of thrombin; activated factors IX, X, XI, and XII; kallikrein; and plasmin. A functional assay of antithrombin (chromogenic assay) is preferred to simply detecting its presence (immunoassay) in plasma. Chromogenic assays determine the amount of functional antithrombin present by adding the test plasma to a reagent containing heparin and excess thrombin or factor Xa, which also contains the chromogen-labeled substrate for thrombin or factor Xa. The more antithrombin present in the test specimen, the less activity of thrombin or factor Xa and thus less color, which is measured spectrophotometrically. A decreased plasma concentration of antithrombin can be caused by widespread coagulation or DIC, decreased hepatic function, or protein-losing enteropathies and nephropathies. Increased antithrombin production has been implicated with acute-phase protein production in cases of acute inflammation and has been suggested to be one of the most accurate prognostic indicators in horses with colic. Antithrombin I complexes in a 1:1 ratio with thrombin, and thrombin–antithrombin complex has been used as an indirect means to evaluate thrombin production/activation during hypercoagulable states.

**Adult Versus Neonatal Differences in Diagnostic Tests**
Clear differences in the reference intervals between adult horses and neonatal foals are present as demonstrated in TABLE 2. The most striking differences are noted when comparing coagulation times of foals younger than 24 hours of age with those of adults. Differences include higher platelet concentrations, increased FDPs, prolonged PT and aPTT, and lower fibrinogen concentrations. With the exception of the platelet concentration, hemostatic parameters in foals are indistinguishable from healthy adult horse values by 1 month of age.

**Conclusion**
Clot formation and dissolution is a dynamic balance of procoagulant and anticoagulant processes necessary in establishing hemostasis while preserving normal tissue perfusion.
Calcium, an essential ion for hemostasis, is used in all reactions of clotting factors, except the first two steps of the intrinsic pathway. The separation of the intrinsic and extrinsic coagulation pathways has greatly facilitated the understanding of secondary hemostasis. However, it is apparent that these two systems function simultaneously to produce thrombin. Tissue factor stimulates the induction of the extrinsic pathway, while factor XII interacts with exposed collagen fibers to initiate the intrinsic pathway. The extrinsic pathway is very rapid, while the intrinsic pathway proceeds at a slightly slower rate. Along with clot formation, activation of fibrinolysis occurs, serving as a “check and balance” to limit detrimental extension of vessel clotting. Normal function of this dynamic process leads to maintenance of vessel patency while simultaneously controlling excessive blood loss. Knowledge of these basic mechanisms of clot formation and the diagnostic means to evaluate coagulation should aid equine clinicians.

**References**

Hemostasis

32. Beasley V. Toxicants that interfere with the function of vitamin K. Veterinary Toxicology. Ithaca, NY: International Veterinary Information Service (www.ivis.org); 1999.

1. Platelet adherence to the vascular endothelium during primary hemostasis is initiated via
   a. platelet glycoprotein.
   b. IgG.
   c. vWF.
   d. thrombosthenin.

2. How does the vascular endothelium prevent platelet adherence in healthy vessels?
   a. exposure of subendothelial collagen
   b. production of the glycosylx

3. Tissue factor combines with factor
   a. IXa
   b. II
   c. XI
   d. VIII, IX, and XII

5. The ability of antithrombin to bind thrombin, thus controlling the rate of clot formation, can be greatly enhanced by
   a. endogenous or exogenous heparin.
   b. TPA.
   c. plasmin.
   d. thrombomodulin.

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6. During fibrinolysis, plasmin is responsible for the proteolytic digestion of
   a. factors IX, X, XI, and XII and thrombin.
   b. factors V, VIII, and XII; fibrin; and prothrombin.
   c. TPA and factors V, VII, IX, and X.
   d. plasma proteins C and S, TPA, and factors II, VIII, and XII.

7. What are the two most important endogenous anticoagulants?
   a. protein C and antithrombin
   b. fibrinogen and plasmin
   c. thrombin and Christmas factor
   d. factor VII and vWF

8. Functional assessment of the intrinsic and common pathways of secondary hemostasis is

   best determined by
   a. PT and ACT.
   b. ACT and PTT.
   c. thrombin time and PT.
   d. PTT and aPTT.

9. During clot dissolution, which of the following tests best quantifies the activity of plasmin?
   a. D-dimer
   b. FDP
   c. PTT
   d. antithrombin

10. A deficiency of vWF, resulting in coagulopathy with a normal platelet concentration, PT, and aPTT, can be attributed to
    a. factor VIII deficiency.
    b. prekallikrein deficiency.
    c. platelet adhesion.
    d. vitamin K deficiency.