**New Insight into the Practical Diagnosis of Bleeding Disorders**

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**Frequently Asked Questions**

**Q: How long is a sample good in a citrate tube?**

A: It is most important to collect a sample into a citrated tube by excellent venopuncture techniques and mix at a ratio of 9:1 for blood to citrate (3.8 or 3.2%). While in the past it was thought essential to immediately chill and analyze a sample, more recent clinical studies suggest that a sample can stay at room temperature for several hours. For the IDEXX Coag Dx™ Analyzer, a citrated sample should not be chilled and also not be separated; *(Note: Chilling can affect/slow the IDEXX Coag Dx test.)* This is a great advantage over the samples that are sent to the laboratory. Furthermore, one can still separate the citrated plasma and submit it for further factor analysis.

**Q: Can blood be drawn from two sites for coagulation testing?**

A: Certainly blood can be drawn from different sites and at different times. Nevertheless, the samples should be collected prior to treatment whenever possible and with the best technique. When using a BD Vacutainer®, I prefer to fill a serum tube first to remove the tissue factor from the venopuncture and then set up citrate and EDTA tubes. Immediate mixing of tubes is important to prevent platelet and coagulation factor activation.

**Q: When drawing blood on a dog with a suspected coagulation disorder, we want to get a good sample and using the jugular and a larger gauge needle is important in that, yet drawing from the jugular with a 20G needle may be a recipe for disaster. What is your recommendation in clinical practice on taking a sample with a suspected bleeding disorder, i.e., idiopathic thrombocytopenic purpura (ITP) or rodenticide exposure?**

A: You make a good point that a proper sample is crucial in getting meaningful results. In animals with bleeding risk, I still prefer to use the jugular vein with a 22G needle and Vacutainer system if possible. Remove the animal’s collar and, if needed, carefully clip the venopuncture site. Properly positioning the animal’s neck area will facilitate the visualization of the vein. Holding off the venopuncture site for 5–10 minutes following collection generally provides the local hemostasis to allow for adequate clotting.

**Q: How was the Coag Dx Analyzer validated for dogs and cats and what were the results?**

A: The IDEXX Coag Dx Analyzer is based upon a similar well established human instrument and the Synbiotics SCA2000. The IDEXX Coag Dx offers some database improvements (allowing docking to the IDEXX VetLab® Station), but otherwise is identical to the earlier SCA2000 veterinary version. The SCA2000 has been validated at the University of Pennsylvania (Penn Vet); and other institutions and practical experience over the past decade has shown its validity. Moreover, the new IDEXX Coag Dx Analyzer has been evaluated by IDEXX and in the clinical practice seems to perform equally well.

**Q: Is there a need to repeat the test results from the IDEXX Coag Dx Analyzer in a reference laboratory to confirm results, or in other words, how accurate is it?**

A: Based upon our studies at Penn Vet and our clinical experience, the point-of-care instrument performs as well as a laboratory coagulation test. In fact, separating plasma and shipping is prone to introduce errors. Because of the ease of handling of the sample and lack of preparing plasma and shipping samples frozen, the results from the Coag Dx Analyzer can be trusted equally as much as reference laboratory results. Major problems may be caused by an inappropriate ratio of blood-to-citrate (has to be 9:1) and collecting blood too slowly from a small vessel or heparinized catheter.

**Q: How long can the citrated blood sit at room temperature before running the IDEXX Coag Dx Analyzer?**

A: Citrated blood can be kept at room temperature for up to 2 hours without affecting the test results of the IDEXX Coag Dx Analyzer. In contrast, when using noncoagulated blood with its whole blood cartridges, samples need to be tested immediately just like for the ACT.

**Q: Are you using nonanticoagulated cartridges with fresh whole blood, and if yes, what for?**

A: While I have used nonanticoagulated cartridges when evaluating these point-of-care instruments, I prefer to use citrated samples. This assures the most accurate results because I do not have to rush to place the nonanticoagulated blood into the cartridge, which allows me to run prothrombin time (PT) and activated partial thromboplastin time (aPTT), and even collect the plasma for von Willebrand factor (vWF) or coagulation factor analysis. There are small citrate tubes that permit small sample collection with the correct ratio.
Q: What are typical results with rodenticide poisoning?
A: A bleeding dog poisoned by anticoagulant rodenticides has massively prolonged PT as well as aPTT; they are generally >3x times normal. However, the thrombin time (TT) is normal in rodenticide poisoning while the TT is often prolonged with liver disease and disseminated intravascular coagulation (DIC).

Q: What are typical results with liver disease?
A: Hepatopathies cause varied forms of coagulopathies because practically all coagulation factors are synthesized in the liver and bile acids are needed for intestinal vitamin K absorption. Hence PT and aPTT can be variably prolonged. Unfortunately, there is no good correlation between the severity of the hepatopathy, degree of coagulopathy based upon PT and aPTT and actual bleeding tendency when performing a biopsy. Therefore, the coagulopathy may be treated with parenteral vitamin K administration and may need to be corrected with fresh frozen plasma prior to performing a biopsy (blood products should be available).

Q: When suspecting a bleeding tendency, would it be appropriate to start off with an aPTT test and then follow up with a PT test?
A: Indeed the aPTT test covers all coagulation factor deficiencies except for Factor VII (FVII). Hereditary FVII deficiency has been reported in several canine breeds including Beagles, Scottish Deerhounds, and Alaskan Klee Kai, causing a mild to moderate bleeding tendency. Furthermore, some hepatopathies may affect the PT more than the aPTT. Ideally, both tests are initially performed simultaneously. If a rodenticide poisoning is suspected, one may also consider testing the PT first and use the PT to monitor the treatment response.

Q: What about dogs on omega-3 fatty acids or vitamin E? Do these supplements have a significant effect on clotting function? And is it necessary to stop these medications prior to surgery/dental extraction/biopsy?
A: A normal amount of an approved supplement will not affect normal hemostatic function in vivo, although in vitro platelet aggregation studies are readily changed by fatty acids such as arachidonic acids. However, any (inappropriate) supplement in excessive amounts may affect some laboratory test results and may even exaggerate bleeding.

Q: What is the treatment for immune-mediated thrombocytopenia (ITP)?
A: Treatment of bleeding disorders was not precisely the topic of this Webinar, but it certainly follows after a diagnosis has been reached and is covered in more detail in another Webinar. ITP may be primary or secondary and thus triggers are removed (many drugs) or treated (doxycycline and other agents for tick-borne diseases). The immune component is treated with prednisolone. In cases of severe thrombocytopenia, vincristine, infused strictly intravenously once, may be added to facilitate platelet release from megakaryocytes and hamper macrophage destruction in the spleen and other organs. In cases of life-threatening bleeding, platelets in the form of fresh (not chilled) whole blood or platelet concentrates should be used, while cases of anemia are corrected with packed red cell transfusions. Other agents, such as cyclosporin and human intravenous immunoglobulin, may be tried but have not been studied extensively and are very expensive.

Q: When does the use of heparin flush affect clotting ability?
A: Whenever blood is collected from a catheter, there is a concern that the heparin may affect the sample, even when removing a 2–3ml sample first (which can be reinfused). Furthermore, if the heparin flush was not appropriately diluted or if heparin concentrate has been used accidentally, anticoagulation of the entire (particularly small) animal may occur. It should also be noted that while the PT is very sensitive to heparin exposure, the PT changes less drastically. Moreover, only unfractionated heparin affects the aPTT and PT while low-molecular-weight (LMW) heparin does not. LMW heparin products may be given at a standard dose or its effects can be assessed by a tenase assay in specialized laboratories or by thromboelastography.

Q: In blood banking, when selecting donors, do you include any screening for inherited bleeding disorders? Do you exclude certain breeds who may be predisposed to these disorders?
A: We generally do not screen our donors for hereditary bleeding disorders, but we would examine any feline or canine donor if excessive bleeding is observed when collecting blood. Moreover, we would screen donors of breeds known to have bleeding problems at risk of being afflicted with a certain hereditary disease, such as Doberman Pinschers for von Willebrand disease (vWD) or male German Shepherds for hemophilia.

Q: Is cryosupernatant effective in the treatment of rodenticide poisoning?
A: Anticoagulant rodenticides deplete vitamin K and thereby inhibit the carboxylation of Factor II (prothrombin Factor II, VII, IX, and X). Because cryoprecipitate only contains fibrinogen, Factor VIII and von Willebrand factor in increased amounts, cryopoor plasma, or cryosupernatant, it is an excellent source of the vitamin K-dependent coagulation factors and thus the preferred blood product to preserve other blood products for other patients.

Q: How do you know if you should use a higher or lower dose of vitamin K1 with poisoning?
A: The actual rodenticide product and amount ingested may give a clue to how long the anticoagulating effects of
the rodenticide will last. While warfarin lasts only a couple of days, others may last for weeks. Furthermore, animals may be reexposed if the poison has not been found and removed. Generally, after the PT and aPTT normalize and bleeding has subsided and healing occurred, the vitamin K is withdrawn and a PT test is repeated 2–3 and 5–7 days thereafter. If a prolongation is observed, the vitamin K is reinstituted for another week and testing is repeated.

Q: I was presented with a 4-month-old kitten with blood dripping from its mouth with an Hct of 10 and Hgb of 3. We have controlled it with prednisone. Apart from the need to withdraw slowly, what can I expect in the future? Also, how concerned should I be about ever vaccinating this kitten? It is FeLV/FIV-negative.

A: I am assuming you made a diagnosis of thrombocytopenia based upon a blood film evaluation. The thrombocytopenia may be immune-mediated or caused by an infection. Even when immune, it may have been induced by an antigen, such as an infection, drug or vaccine, and thus may reoccur when reexposed. Because this is likely a secondary ITP or other cause of thrombocytopenia, relatively rapid tapering of prednisolone (some cats may not respond to prednisone but do so to prednisolone) is likely warranted within less than a month with follow-up testing. At 4 months of age, this kitten is likely well vaccinated for a year. Because of the lack of a clear association to a vaccine-induced ITP I would booster the cat when needed, but I would also get a platelet count before and 10 days afterwards and monitor the cat for bleeding. Other options may be hereditary (hemophilia in males and vitamin K dependent coagulopathy in Devon Rex) and acquired (rodenticide) coagulopathies.

Q: What is your opinion on nonsteroidal anti-inflammatory drugs (NSAIDS) and their effect on clotting disorders/times?

A: NSAIDS, such as aspirin, will affect platelet function and thus the buccal mucosal bleeding time (BMBT), but not the PT and aPTT. Their bleeding tendency is mild, except they can cause ulcers and thereby bleeding, or exaggerate an underlying bleeding tendency such as von Willebrand disease or ITP.

Q: What is the PIVKA test? Is it diagnostic for rodenticide toxicity and should it be used in practice?

A: The PIVKA test implies that it measures proteins induced by vitamin K antagonism/absence, and thus the name PIVKA. However, the PIVKA test does not measure these proteins, but only the deficiency of the coagulation factors in the extrinsic and common coagulation pathway. It represents a PT test and, therefore, is not specific for rodenticide poisoning. As it is nothing more than a PT test, I do not recommend using this test; it only adds unnecessary costs (has been completely abandoned in human medicine).

Q: If a cat with a hepatopathy has a normal coagulation parameter, is it safe to perform a blind liver biopsy?

A: It is difficult to predict the degree of bleeding from a liver biopsy. The better visualization of hepatic blood vessels by ultrasound can prevent the accidental laceration of major hepatic vessels. As bleeding may still occur, blood products should be available and the risk needs to be explained to the owner.

Q: What are the PT and aPTT reference ranges for different species for the IDEXX Coag Dx Analyzer?

A: Normal ranges have been established for the dog, cat and horse. It should be noted that while the normal PT is short and similar to that of a PT from a reference laboratory, the normal aPTT is much longer and more like that of an activated clotting time.

Q: Can cartridges be used on both the old (SCA2000) and the new IDEXX instrument?

A: Cartridges are no longer supplied by Synbiotics, however, they are available through IDEXX. These new cartridges work for both the old SCA2000 and the new IDEXX Coag Dx Analyzer.

Q: Is it true that some rodenticide-poisoned dogs also have thrombocytopenia?

A: Indeed rodenticide-poisoned animals may also have moderate to severe (as low as 25,000/ul) thrombocytopenia which is thought to be caused by direct toxicity of the rodenticide on the bone marrow.

Q: Do I need to worry about bleeding in Cavalier King Charles spaniels (CKCSs) with thrombocytopenia?

A: While CKCSs do have a tendency for megaplatelets and thrombocytopenia, they do not show an increased bleeding tendency in vivo.

Q: Is it true that the aPTT is normal in vWD in dogs?

A: Correct. Deficiency of canine vWF does not affect the aPTT, presumably due to the fact that sufficient unbound Factor VIII remains in circulation.

Q: Is the BMBT sensitive to discovering vWD in an emergency setting?

A: Yes. When a normal platelet count is assured, a prolonged BMBT is suggestive of canine von Willebrand’s disease type I and III. A specific vWF analysis by an ELISA test on citrated or EDTA blood should be subsequently performed to confirm the vWF deficiency, as thrombopathias will also prolong the BMBT.