Hematology Techniques WetLab
North Central Texas VMA
August 29, 2010

Learning Objectives:

1. Observe the bone marrow aspiration collection process, using the "IM" method at the humeral head. Needle placement can be also used for intraosseous fluid administration.
2. Prepare bone marrow slides for cytology, confirm that the sample is adequate, and understand how to submit them to an outside lab for interpretation for best results.
3. Become familiar enough will the process of collecting plasma from dogs to be able to harvest and store plasma at your clinic for future use.
4. Become familiar enough with the process of blood typing dog and cat blood and performing major cross-match to be able to perform these tasks at your clinic.
5. Perform Activated Clotting Time in the dog or cat.
6. Performing Buccal Mucosal Bleeding Time in the dog.
7. Become familiar with using blood filters for cat transfusions and hemotap filter spikes.

PART ONE: BONE MARROW ASPIRATION DEMO.

Steps Instructors will be taking to aspirate bone marrow from a dog, for lab participants to confirm adequate sample and practice preparing bone marrow cytology slides.

1. *Preparation for bone marrow aspirate:*

    a. **Sedation.** Dog is placed under deep sedation with a protocol such as Domitor-butorphanol, acepromazine-oxymorphone, etc. General anesthesia can be used, but is not usually necessary.

    b. **Surgical prep.** A 2-3 inch square of hair is clipped over the right humeral greater tubercle, and prepped as if prepping for surgery, then draped. If Doc is left handed, use the left humerus, and substitute "left" for "right" humerus throughout this lab.

    c. **Nonsterile supplies** are gathered and available.
        i. Clippers
        ii. 18 gauge needle to coat the syringe with anticoagulant.
        iii. Anticoagulant – EDTA or Heparin.
        iv. Sterile scrub supplies – cotton balls or gauze sponges, surgical scrub, isopropyl alcohol.
        v. Petri dish or coffee mug for bone marrow sample.
        vi. Glass slides, pipettes and paper towels for preparing bone marrow slides.
        vii. EDTA blood taken from patient in last 24 hours – this must be sent to the lab with the bone marrow sample to the bone marrow can be interpreted.
        viii. Extra sterile supplies are gathered in case needed.

1. 18g needles.
2. 10-12cc syringes.

d. **Preparing the Sterile Field.** Sterile gloves in the appropriate size are opened and the paper covering is used as a sterile field.
   i. Sterile items are placed on the sterile field for use:
      1. 10-12cc syringe.
      2. Bone marrow aspiration needle.
      3. Injectable lidocaine for local block.
      4. 3cc syringe with 22g x 5/8" needle for lidocaine block.
      5. #11 surgical blade.
   ii. Check the bone marrow needle for sharp tip, proper seating of the stylet and attachment of the stylet cap. Also make sure the stylet moves freely into and out of the needle.
   iii. Surgical gloves are donned.
   iv. Assistant holds bottle of lidocaine so that Doc can draw up appropriate amount of lidocaine for local block in the 3cc syringe.

2. *Bone marrow aspiration.*

a. **Find your spot**. Doc grasps the right humerus near the elbow with the left hand, rotating the elbow toward the chest wall, to make the greater tubercle of the humerus more accessible.

b. **Lidocaine block.** The "flat spot" on the top of the greater tubercle is palpated, and lidocaine for local block is injected into the skin, subcutaneous tissues and periosteum.

c. Stab incision. Using the #11 blade, a single stab incision is made in the blocked tissue, all the way down to bone.

d. **Introduce bone marrow needle.**
   i. Bone marrow aspiration needle is introduced into the stab incision, until it rests on bone, with the needle shaft parallel to the long axis of the humerus, directed into the center of the marrow cavity.
   ii. The bone marrow needle is rotated slowly but firmly back and forth until it enters the cortex. The needle can easily slip at this time causing trauma, so take care when performing this step.
   iii. As soon as the needle is seated in the cortex, then it is advanced into the marrow cavity in a screwdriver-like motion, until well seated in the marrow cavity. You will not be able to "feel" the needle drop into the marrow cavity.
   iv. When you suspect you have reached the marrow cavity, check the needle to make sure it is firmly seated in bone, with no wobbling.
   v. The bone marrow needle can now be used for either bone marrow sampling or intraosseous fluid administration.

e. **Preparing the sterile aspiration syringe.**
    i. Assistant holds 18g needle so that Doc can remove the hub cover and attach the 10-12cc syringe.
    ii. Assistant continues to hold 18g needle cover while Doc removes the sterile syringe with attached needle.
    iii. Assistant holds anticoagulant bottle while Doc aspirates 1-2cc from the bottle.
    iv. Doc pulls the syringe all the way to the largest cc marking to coat the inside of the syringe with anticoagulant
    v. Anticoagulant is then squirted into the coffee mug or petri dish.

f. **Aspirating bone marrow.**
    i. The needle is detached from the syringe, and the anticoagulant coated syringe is attached to the bone marrow aspiration needle in place.
    ii. The syringe plunger is quickly aspirated until bone marrow appears in the hub, and then immediately released, so that 1-2cc of marrow is in the syringe. More volume will just dilute your marrow sample with blood.
    iii. Marrow sample is immediately squirted into the anticoagulant in the coffee mug or petri dish, and checked for spicules.
    iv. If no marrow appears in the hub, the needle must be removed, rechecked to make sure it is not plugged, and replaced.
    v. If no spicules are present in the marrow, the needle must be removed, rechecked to make sure it is not plugged, and replaced.

3. *Confirming adequate bone marrow sample and removing the needle.*
    a. If spicules are present in the bone marrow sample, cytology slides are made, stained and examined to confirm presence of bone marrow.
    b. If slides confirm adequate sampling, the bone marrow needle can be removed.

4. *Bone marrow core biopsy – not done in this wetlab.*
    a. If multiple attempts at bone marrow aspiration from different bones are unsuccessful, bone marrow core biopsy is indicated.
    b. Core biopsy is performed as aspiration, except that as soon as the bone cortex is fully penetrated, the stylet is removed and the cap replaced.
    c. The needle is then advanced by rotating in a single direction, at least 1-2 more inches.
    d. The needle is then forcefully rotated without advancement in an attempt to break the biopsy loose from the marrow tissue.
    e. The needle is removed, the cap removed, and the style passed backward from the pointed end to produce the core biopsy from the needle attachment hub end of the bone marrow aspiration needle.
    f. This process should be repeated until a core biopsy can be produced.
    g. The core biopsy can be rolled onto a glass slide to prepare cytologies to confirm adequate sample, prior to putting the biopsy in formalin and submitting to the lab for histopathology.

PART TWO: PREPARING BONE MARROW CYTOLOGY SLIDES

5. Find all materials on demo table, and stains and microscopes are in the microscope lab.
    a. Bone marrow in anticoagulant.
    b. Glass slides.
    c. Pipettes.
    d. DiffQuick Stain.
6. Use pipette to aspirate 2-3 spicules from marrow sample, and place on a glass slide.
7. Tilt the slide so that the blood runs off the bottom edge of the slide, while using the pipette to push the spicules back up to the top of the slide.
8. Once the excess blood is off the slide, make cytology preps using both vertical and gentle horizontal pull-apart techniques. Lymphoid cells can be especially susceptible to trauma, so be gentle to prevent damaging marrow cells.
9. Stain 1-2 slides to confirm presence or marrow.
10. Submit both stained and unstained slides with the CBC for cytology interpretation.
11. Ship formalin fixed slides in a separate box from cytology slides, as formalin fumes can damage cytology slides and prevent proper staining.

PART THREE: PLASMA COLLECTION

12. Gather materials:
    a. Clippers
    b. Surgical scrub materials
        i. Cotton balls or gauze sponges
        ii. Surgical scrub
        iii. Isopropyl alcohol
    c. White tape to secure blood bag during blood draw.
    d. Purple top tube for CBC on your dog and 3cc syringe to take blood, or CBC results may be provided.
    e. Plasma bag with anticoagulant.
    f. Sharpie pen for labeling.
    g. Scissors to cut tubing after knotted.
13. Check CBC results on dog to make sure blood collection is safe.
14. Label plasma bag with patient name and date of collection.
15. Place the pet in either lateral recumbency or sternal recumbency for venipuncture, according to your preference.
16. Clip and surgically prep the jugular vein for venipuncture.
17. Attach the blood collection bag with white tape to a place lower than the dog, where it can be visually monitored for continued blood flow.
18. Uncap the blood collection needle of the plasma bag, and place the needle in the jugular vein, keeping it in place until the bag is full.
19. Remove the needle from the jugular vein, and apply pressure with cotton ball or gauze for 1 minute. Confirm hemostasis after the cotton ball or gauze is removed.
20. Crimp or tie a knot in the blood collection line, and remove the needle from the tubing above the crimp/knot by cutting the line.
21. Separate packed cells from plasma by one of the following methods within 2 hours of collection to produce fresh frozen plasma.
    a. Centrifuge at a blood bank.
    b. Manual separation of packed cells and plasma after allowing to separate overnight.
22. Freeze plasma immediately after separation, and use within 1 year.
23. Store packed cells in the refrigerator and use within 30 days.

PART FOUR: BLOOD TYPING AND CROSS-MATCH. Each lab participant will do either a feline blood type, a canine blood type or a cross-match (either species). Cross Matches will be performed in teams of 2 participants per test.

24. *Find extra supplies on the supply table. Test kits will be on each lab table.*
    a. EDTA dog blood for typing and crossmatch.
    b. EDTA cat blood for typing.
    c. Dog serum for crossmatch.

25. *Dog and Cat Blood Typing.*

    a. **How the Test Works.** The RapidVet-H assays are based on the agglutination reaction that occurs when an erythrocyte which contains an antigen (Canine DEA 1.1, Feline A or B) on its surface membrane interacts with a murine monoclonal antibody proven specific to which is lyophilized on the Test Card.

    b. **False positives.** A certain number of canine patients exhibit auto-agglutination of varying degrees due to serum factors that cause agglutination of the patient's own red cells. If a patient exhibits this under test conditions, it will not be possible to definitively type this patient without separating the serum and serially washing the remaining red cells before performing the test. *RapidVet-H provides a separate reaction card for use to screen for such patients.*

26. *Canine Blood Typing.*

    a. **Reagents and Materials for Canine Blood Typing:** This test kit contains the reagents and materials listed below. Store materials upright and at room temperature or in the refrigerator, except for controls which must be refrigerated.
        i. ***Auto-Agglutination Saline Screen Card*.** This card has 3 visually defined wells (1 per dog tested).
        ii. ***Agglutination Test Cards.*** Each card has 3 visually defined wells (1 per dog tested) identified as "DEA 1.1 Positive Control", “DEA 1.1 Negative Control” and "Patient Test”. Store at room temperature or in a refrigerator.
        iii. ***1 Bottle Diluent*.** The clear plastic bottle contains 0.02 mol/L phosphate buffered saline (PBS) at pH 7.4.
        iv. ***1 Bottle Positive Control (optional).***
        v. ***1 Bottle Negative Control.***
        vi. ***Pipettes and Stirrers*.**

    b. **Canine Blood Type Procedure.**

        i. ***Adjust sample for severe anemia***. If the dog being tested has PCV <10%, the sample should be spun down and sufficient plasma removed by pipette so that the hematocrit is increased to >15%.

ii. ***Draw blood*** from the patient into a lavender tube coated with or containing EDTA as an anticoagulant. The assay requires only 100 **µ**l whole blood but the tube or syringe should be full so that there is a proper concentration of EDTA.

iii. ***Screening the Patient for Autoagglutination.***

1. Remove the Auto-Agglutination Saline Screen Card from its plastic sleeve and place the card on a flat surface. Write the name/number of the dog and the testing date on the Auto-Agglutination Saline Screen Card adjacent to the well to be used. If all three tests will not be used, save the plastic sleeve for storage.
2. Dispense 1 drop of diluent from the dropping bottle into the well to be used.
3. Aspirate a small amount of patient sample into the pipette and release 1 drop into the well. Using a stirrer and pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of this well for about 10 seconds.
4. A small percentage of ill dogs and of healthy dogs auto-agglutinate. *If agglutination is observed,* ***STOP*** *the test and perform normal cell washing procedures before proceeding.* It may be possible to determine whether the dog is DEA 1.1 positive or negative despite the auto-agglutination if the auto-agglutination is light. If the user proceeds on this basis, the following criteria could be used:
    a. If the appearance in the patient well is the same as in the auto-agglutination well, the dog is likely DEA 1.1 negative.
    b. If the result in the patient well appears as substantially more agglutination than in the auto-agglutination well, the dog is likely DEA 1.1 positive.
5. If auto-agglutination does not occur, proceed to the next step.
6. After the materials on the Auto-Agglutination card have dried, replace the card in its plastic sleeve for future use.

iv. Testing the patient.

1. Remove the Test Card from its plastic sleeve. Save the plastic sleeve and set aside the desiccant bag.
2. Write the name/number of the dog and the testing date on the card and place the Test Card on a flat surface.
3. Dispense 1 drop of diluent from the dropping bottle into each well. The diluent assists in reconstitution of the lyophilized antibody in the control and patient well.
4. Gently swirl the bottles containing the control and the patient sample to resuspend any solid material.
5. Unscrew the top of the control bottle and place it on table in front of the Test Card. Place the cap behind the respective bottle, to avoid cross-contamination.

6. Dispense 1 drop Positive Control into the well marked “DEA 1.1 Positive Control”. Using a new stirrer and pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of the well for about 10 seconds.
7. Dispense 1 drop Negative Control into the well marked “DEA 1.1 Negative Control”. Using a new stirrer end and pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of the well for about 10 seconds.
8. Aspirate a small amount of patient sample into a pipette and release 1 drop into the well marked “Patient Test”. Using a new stirrer and pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of the well for about 10 seconds.
9. Rock the card with a transaxial motion for 2 minutes, being sure that the materials are mixing and "rotating" within each well. Be careful not to cross contaminate.

v. Reading Results.

1. Agglutination is the electrostatic binding of cells and antibodies and thus is reversible. If agglutination seems to appear and then disappear, or if the user is not certain if what is seen is agglutination, this is likely due to a Prozone Effect. This can occur because, as a result of dogs having multiple blood types, there are insufficient DEA 1.1 antigens on the red cell in relation to the concentration of monoclonal antibody. In such instances, add a second drop of diluent and rock the card for an additional 30 seconds before reading the results. This will potentiate the reaction if, and only if, the animal is DEA 1.1 positive.
2. Read the results and note the wells where gross agglutination has occurred.
    a. If the assay was run correctly, visible, gross agglutination should have occurred in the well marked “DEA 1.1 Positive Control”.
    b. If the patient sample shows gross agglutination in the well marked “Patient Test” and there is no auto-agglutination, the patient is DEA 1.1 positive.
    c. If no agglutination is visible in the well marked “Patient Test”, the patient is DEA 1.1 negative.
    d. Any fine, granular appearance developing after 2 minutes should be disregarded in determining the results. The speed of agglutination and the size of the clumps of cells of a DEA 1.1 positive patient may differ from that of the positive control.
3. After the card has been read, take a digital photograph of it for a permanent record. Alternatively, set the card at a 10° angle to allow excess blood to run to the bottom of the wells. Placing the

top of the card on the desiccant bag will accomplish this. After the materials on the card have dried, replace the card in its plastic sleeve for a permanent record.

4. Before replacing the control bottles in the box, tap the bottom of each bottle firmly on the table to cause residual liquid in the dropping tip to fall back into the bottle. Store upright.
5. Dispose of all biological materials, pipettes and stirrers in a biohazard container.

27. *Feline Blood Typing.*

a. **Reagents and Materials for Feline Blood Typing.** Store upright at room temperature or in the refrigerator.

i. Agglutination Test Cards. Each card has 3 visually defined wells, one identified as "Auto-Agglutination Saline Screen" and two wells identified as "Patient Test" – one Type A and one Type B. If the test is run properly, at least one of the wells labeled "Patient Test" will agglutinate. Thus, the test is self-controlled.

ii. **1 Bottle Diluent.** The clear plastic bottle contains 0.02 mol/L phosphate buffered saline (PBS) at pH 7.4.

iii. **Pipettes and Stirrers.** Each polyethylene bag contains 2 plastic pipettes and 3 stirrers.

b. Feline Blood Type Procedure.

i. ***Draw blood*** from the patient into a tube coated with or containing EDTA as an anticoagulant. The assay requires only 150 **µ**l whole blood but the tube should be full or the syringe should be filled so that there is a proper concentration of EDTA.

ii. ***Checking the Cat for Autoagglutination***.

1. Remove the Test Card from its plastic sleeve. Save the plastic sleeve and set aside the desiccant bag.
2. Write the name/number of the cat and the testing date on the card. Place the Test Card on a flat surface.
3. Dispense 1 drop of diluent from the dropping bottle into the well marked "Auto-Agglutination Saline Screen".
4. Aspirate a small amount of patient sample into the pipette and release 1 drop into the well marked "Auto-Agglutination Saline Screen." Using a stirrer and pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of this well for about 10 seconds.
5. If agglutination is observed, **STOP** the test and perform normal cell washing procedures before proceeding. If no agglutination occurs, proceed to next step.

iii. Typing the patient.

1. Dispense 1 drop of diluent from the dropping bottle into each remaining well to be used. The diluent assists in the reconstitution of the lyophilized material.
2. Gently swirl the tube containing the patient sample to resuspend any solid material.
3. Aspirate a small amount of patient sample into the pipette and release 1 drop into each of the 2 wells marked "Patient Test". Using a new stirrer and pressing downward with the flat portion of the stirrer, spread and mix the materials within the **ENTIRETY** of one of the wells for about 10 seconds. Take a new stirrer and similarly spread and mix the materials within the **ENTIRETY** of the other well for about 10 seconds.
4. Add a second drop of diluent to the well marked "Type A". Do not stir the well with a stirrer.
5. Rock the card, using a transaxial motion, **for 1 minute or, if less, until agglutination has occurred in at least one of the "Patient Test" wells**, being sure that the materials are mixing and "rotating" within each well. Be careful not to cross contaminate the wells.

iv. Reading results.

1. The results and note the wells where agglutination has occurred.
2. If the assay was run correctly, visible agglutination should have occurred in at least one of the wells marked "Patient Test."
3. If the patient sample shows agglutination in the well marked Type A, the cat tested has blood group A.
4. If the patient sample shows agglutination in the well marked Type B, the cat tested has blood group B.
5. If the patient sample shows agglutination in both patient wells, the cat tested has blood group AB.
6. Any fine, granular appearance developing after 1 minute should be disregarded in determining the results. It may be possible to determine if the cat is Type A or B despite the auto-agglutination if the auto-agglutination is light.
7. If the appearance of the agglutination in one of the A well or the B well is similar to that seen in the auto-agglutination well, the cat is likely negative for that blood type. If that is true for both the A well and the B well, the procedure should be stopped because the cat cannot be negative for both the A and B blood type.
8. If the agglutination in one of the wells is greater than that in the auto-agglutination well, the cat is likely positive for that blood type. If that is the case in both wells, in light of the rarity of AB cats, it is not possible to reach a conclusion.
9. After the card has been read, take a digital photograph of it for a permanent record. Alternatively, set the card at a 10° angle to allow excess blood to run to the bottom of the wells. Placing the

top of the card on the desiccant bag will accomplish this. After the materials on the card have dried, replace the card in its plastic sleeve for a permanent record.

28. *Cross Matching (Canine or Feline)*

a. **Collect Donor Sample**: 0.5ml EDTA anticoagulated whole blood or whole blood segment sample from previously collected packed RBCs or whole blood.
b. **Collect Recipient Sample**: 1.0 ml serum obtained by centrifuging 2.0 ml whole blood in a serum (red top) tube.
c. **Test Setup.**
   i. Remove one test stand containing 6 tubes, blue top tube, pipette bag and report card from kit.
   ii. Write Donor name/ID on all seven (7) tubes provided.
   iii. Write Recipient name/ID on Yellow Top Reaction **(R)** Tube and Clear Top Reaction **(R)** Gel Tube (yellow-bordered label).
   iv. Insert Blue Top Donor Blood Prep Tube upright into well provided in test stand.

d. **Test Procedure**

   i. Add 10 drops Donor EDTA Sample to Blue Top Donor Blood Prep Tube using clean pipette from kit; cap Blue Top Tube tightly and invert several times to mix thoroughly. Place upright in test stand.
   ii. Transfer 4 drops Recipient serum to Yellow Top Reaction **(R)** Tube using a clean pipette.
   iii. From Blue Top Donor Blood Prep Tube
      1. transfer 2 drops to Yellow Top Reaction (R) Tube using a clean pipette; cap Yellow Top Tube tightly and invert several times to mix thoroughly.
      2. Transfer 2 drops to Green Top Negative **(-)** Control Tube using a clean pipette; cap Green Top Tube tightly and invert several times to mix thoroughly.
      3. transfer 2 drops to Red Top Positive **(+)** Control Tube using a clean pipette; cap Red Top Tube tightly and invert several times to mix thoroughly.

   iv. **INCUBATE:** Let all tubes stand for 5 minutes at room temperature.

   v. Transfer 1 drop from Yellow Top Reaction **(R)** Tube to Clear Top Reaction **(R)** Gel Tube (yellow-bordered label) using a clean pipette. Cap tightly.
   vi. Transfer 1 drop from Green Top Negative **(-)** Control Tube to Clear Top Negative **(-)** Control Gel Tube (green-bordered label) using a clean pipette. Cap tightly.

vii. Transfer 1 drop from Red Top Positive **(+)** Control Tube to Clear Top Positive **(+)** Control GelTube (red-bordered label) using a clean pipette. Cap tightly.

viii. Place Gel Tubes in centrifuge and spin according to the centrifuge chart that follows.

ix. Interpret Clear Top Positive **(+)** Control and Negative **(-)** Control Gel Tubes using the Crossmatch Photo Identifier provided.

1. **NEGATIVE CONTROL**: Clear Top Negative **(-)** Control Gel Tube (green-bordered label) should demonstrate a collection of red blood cells at the **bottom** of the gel column.
2. **POSITIVE CONTROL**: Clear Top Positive **(+)** Control Gel Tube (red-bordered label) should demonstrate an agglutination of red blood cells at the top of the gel column or a dispersion of red cells mid matrix and above.
3. **IMPORTANT: If Positive (+) and Negative (-) controls do not react as stated above, DO NOT proceed with the interpretation of test.**

x. CROSSMATCH INTERPRETATION: Interpret reaction in Clear Top Reaction **(R)** Gel Tube (yellow-bordered label) using the Crossmatch Photo Identifier provided. Record results using report card provided in kit.

1. A **POSITIVE CROSSMATCH** indicates the Recipient is at risk for demonstrating a transfusion reaction. **DO NOT TRANSFUSE USING THIS DONOR.**
2. A **NEGATIVE CROSSMATCH** indicates the Recipient is likely NOT at risk for demonstrating a transfusion reaction from the Donor.
3. Test results might be affected by the age of the cells used. Stored blood might exhibit a weaker reaction than that shown in the Photo Identifier.
4. **NOTE: CROSSMATCHING IS DONE IN ADDITION TO, AND DOES NOT REPLACE, BLOOD TYPING. Transfusions involving incompatible BLOOD TYPES will result in the activation of alloantibodies which may cause life-threatening reactions, or the production of antibodies which may cause serious complications in subsequent transfusions. In addition, the lifespan of incompatible RBCs will be shortened, increasing the need for further transfusions.**
5. **Oxyglobin and severe hemolysis**

PART FIVE: ACTIVATED CLOTTING TIME WITH KAOLIN TUBES

1. Gather materials:
    a. Kaolin ACT tube.
    b. 3cc syringe.
    c. Clock or watch with second hand.
2. Warm the kaolin tube to body temperature, and keep warm. Can use warming block or body heat.
3. Draw whole blood without anticoagulant from a peripheral vein into a 3cc syringe, from dogs that may have coagulopathy.
4. Put the exact amount of whole blood required by tube size immediately into the kaolin ACT blood tube – usually 2 ml.
5. Invert once every 15-30 seconds.
6. The first sign of clot, no matter how small, is the ACT.
7. Normal is less than 2 minutes.

PART SIX: BUCCAL MUCOSAL BLEEDING TIME

1. Gather materials:
    a. Bleeding time device
    b. Platelet count and ACT results.
    c. Roll gauze if muzzle is needed.
    d. Paper towel or gauze to dab blood drips.
    e. Watch or clock with second hand.
2. In order to do this test safely, confirm that:
    a. platelet count is >50,000/ul
    b. ACT is normal
    c. The patient has no Petechiae or Ecchymoses
    d. The patient is not in DIC.
3. Lift the lip to expose the buccal mucosa. If the dog is crabby or wiggly, apply a gauze muzzle which keeps the lip elevated.
4. Remove the Bleeding Time device safety tab.
5. Place the device against the buccal mucosa where the blades come out when the trigger is pulled.
6. Push the device trigger button.
7. Dab dripping blood as needed – every 15 seconds or so – but do not disturb the clot or touch the cut. Do not allow blood to drip into the mouth, or the patient may lick the cut/clot and invalidate the test.
8. When bleeding stops, you have BMBT (Buccal Mucosal Bleeding Time).
9. Normal is 2-4 minutes.
10. 5 minutes isn't worrisome.
11. Prolonged BMBT indicates primary hemostatic defect.
12. Check your patient in 15 minutes or so for rebleeding which would indicate secondary hemostatic defect.