Bone marrow Examination :

Bone marrow, or myeloid tissue, is a soft, gelatinous tissue that fills the cavities of the bones. Bone marrow is either **red or yellow**, depending upon the preponderance of hematopoietic (red) or fatty (yellow) tissue.

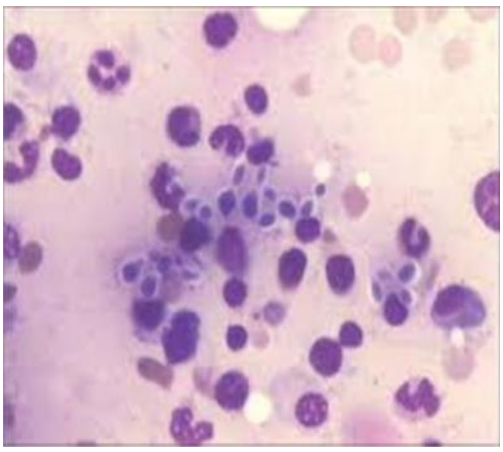
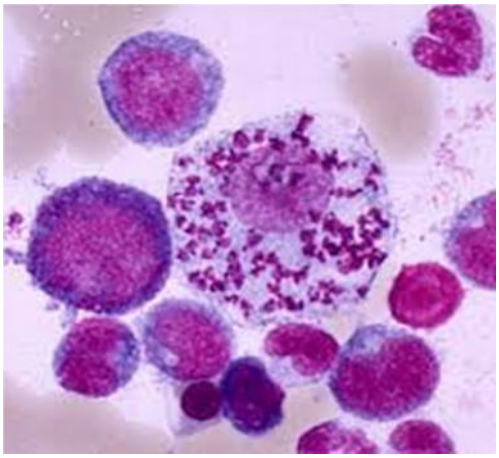
In humans the red bone marrow forms all of the blood cells with the exception of the lymphocytes which are produced in the marrow and reach their mature form in the lymphoid organs. Red bone marrow also contributes, along with the liver and spleen, to the destruction of old red blood cells. **Young animals** have active (red) marrow throughout most skeletal bones. Active marrow recedes from long bones as adulthood is reached, **because** the bone marrow space expands faster than the blood volume as the animal grows. **Active marrow** remains in the flat bones (vertebrae, sternum, ribs, and pelvis) and proximal ends of the humerus and femur in adults

Yellow bone marrow serves primarily as a storehouse for fats but may be converted to red marrow under certain conditions, such as severe blood loss or fever. At birth and until about the age of seven in man, all human marrow is red, as the need for new blood formation is high. Thereafter, fat tissue gradually replaces the red marrow, which in adults is found only in the vertebrae, hips, sternum, ribs, and skull and at the ends of the long bones of the arm and leg.

It is indicated when peripheral blood abnormalities are detected as:

1. **Unexplained continuous decrease in the number of blood cells as** Persistent neutropenia; unexplained thrombocytopenia; poorly regenerative anaemia or a combination of all(pancytopenia).
2. **Unexplained continuous increase in the number of blood cells or proliferative abnormalities as** persistent thrombocytosis; leukocytosis; abnormal morphology of RBCs or unexplained presence of immature cells in the blood.

3. Stage of a neoplastic condition as lymphomas and mast cell tumors.
4. Estimate the adequacy of body iron stores.
5. Search for occult disease in animals with fever of unknown origin or unexplained weight loss.
6. Useful in determining the cause of a hyperproteinemia when it occurs secondarily to multiple myeloma, lymphoma, leishmaniasis, and systemic fungal diseases.
7. Looking for occult neoplasia and Looking for organisms that cause systemic infection as: Histoplasma– Leishmania – Cytauxzoon

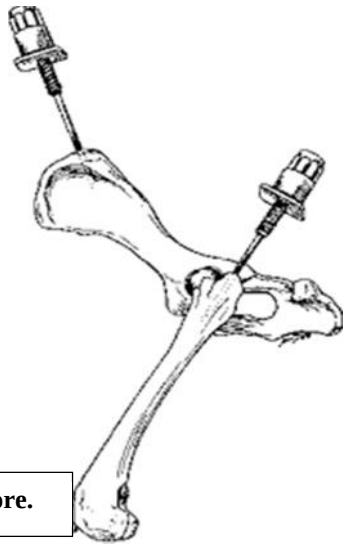
Technique for bone marrow biopsy:

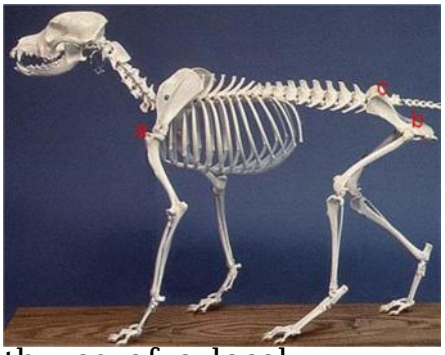
Site of collection:

- **Dogs:** The sites that are most accessible for bone marrow aspiration in the dog **are the proximal humerus (a), proximal femur (b) and the wing of the ilium (c), approached either from the dorsal crest or lateral face.** The wing of the ilium may be "out of reach" of the bone marrow needle in obese or large dogs. The same applies to the proximal femur**. Less commonly, a rib or the sternum (breast bone) may be aspirated.**
- **Cat:** The easiest sites from which to obtain bone marrow **in the cat are the proximal femur and proximal humerus.**
- **Large animals: The sternum, third, fourth or fifth sternebra, a short biopsy needle with an adjustable guard should be used to avoid penetration and damage to the thoracic cavity.** Bone marrow may be collected from the proximal part of ribs also.

Rosenthal aspiration needle (16g x 1 - 5/16").

Jamshidi needle for obtaining a core.





Collection sites.

(A) The greater tubercle of the proximal humerus.

(B) The trochanteric fossa of the proximal femur.

C) The iliac crest of the pelvis.

Preparation of Animal

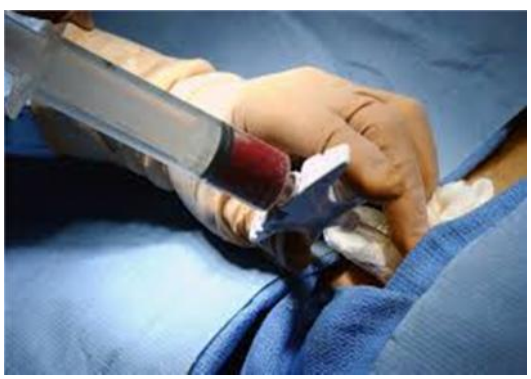
Most bone marrow aspirates can be obtained with use of a local anesthetic and mild sedation. The position of the patient during the procedure depends on the site being aspirated or biopsied.
Hair is shaved from the site, and the skin is scrubbed as for a sterile procedure. A local anesthetic, such as lidocaine, is injected into the area to be aspirated so that the site becomes numb, which minimizes the patient's discomfort.

Description of Technique

Once the site has been clipped and prepared, a small incision is made in the skin The biopsy needle is inserted and, with firm pressure and a clockwise-counterclockwise motion, inserted through the cortical bone. The stylet is removed and a syringe applied (2-20ml depending on personal preference).
Negative pressure is applied to the syringe until blood appears in the tip of the syringe. Up to 0.5ml fluid is collected. A sample of marrow is aspirated into the syringe attached to the bone marrow needle and submitted for analysis; few drops of EDTA are added to the syringe to prevent clotting of the bone marrow aspirate. EDTA tubes should also be available.
Once a good sample has been obtained, the needle is removed, and the small incision in the skin is sutured, glued together, or left to heal by itself. The biopsy needle used to aspirate bone marrow must have a removable stylet that remains in place until bone

marrow cavity is entered to prevent obstruction of the needle lumen with bony tissues.

Several air-dried smears are prepared within seconds of collection. Larger volumes of fluid can be preserved in EDTA, but cell preservation is not as good as with fresh air-dried smears. Even with EDTA, smears should still be prepared within 1-2 hours.



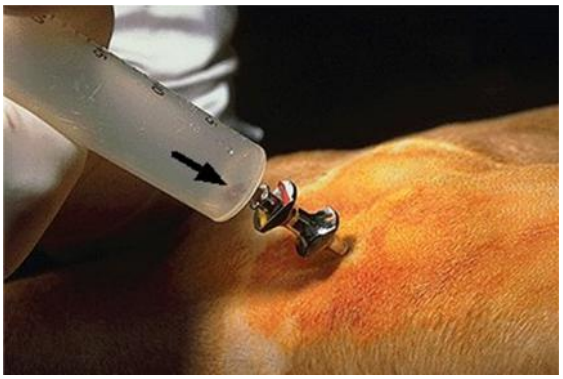
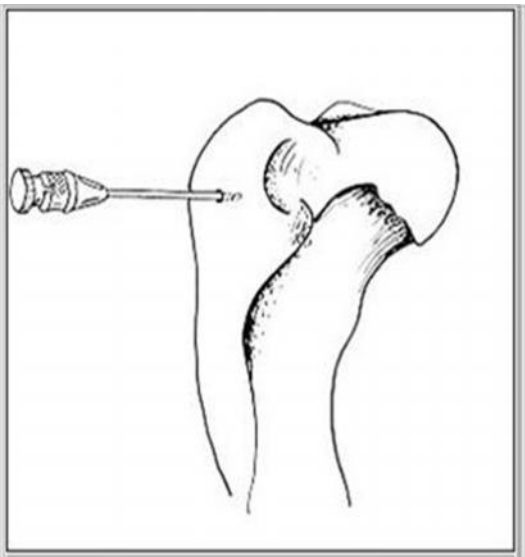


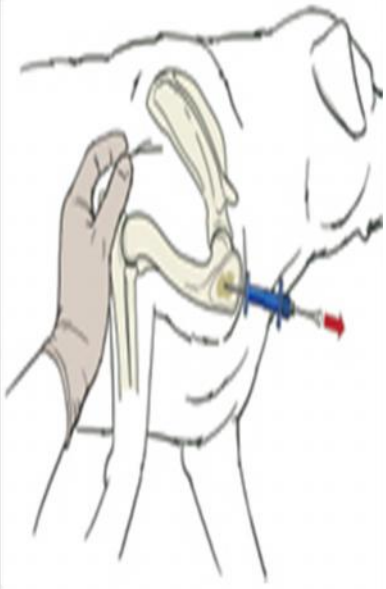
Figure. Rapidly apply suction (8 to 10 ml). As soon as a flash of blood appears in the hub of the syringe, immediately release the suction to prevent hemodilution, a common cause of poor-quality samples.

A CBC is often submitted along with the marrow sample to provide the pathologist with a more complete picture of the clinical condition.



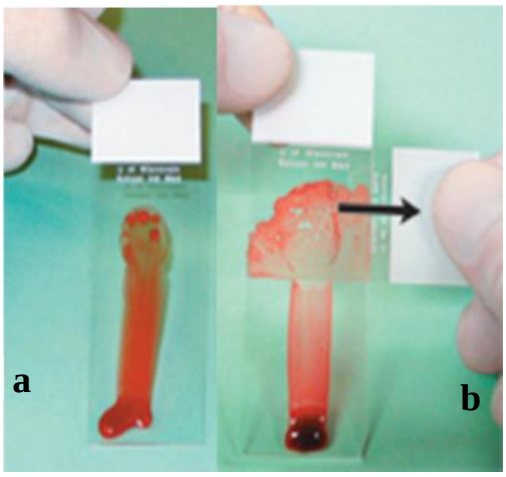
Bone marrow aspiration from the proximal humerus. (From Morgan RV: Selected diagnostic and therapeutic procedures. P. 17. In Morgan RV (ed): Handbook of Small Animal Practice. 3rd Ed. WB Saunders, Philadelphia, 1997, with permission.)

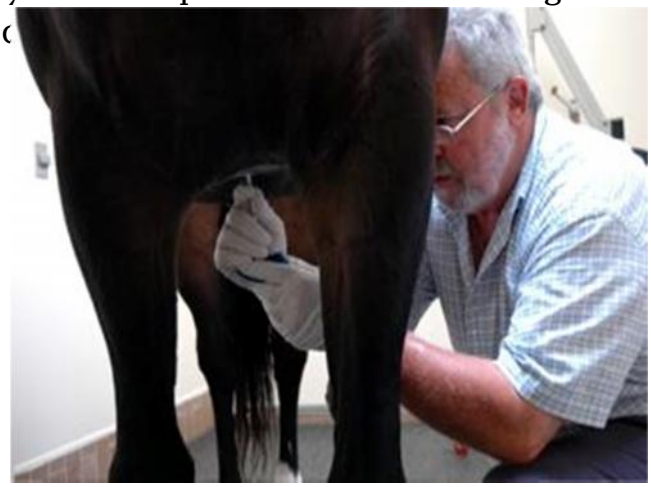
Bone marrow aspiration from a rib. (From Morgan RV: Selected diagnostic and therapeutic procedures. P. 17. In Morgan RV (ed): Handbook of Small Animal Practice. 3rd Ed. WB Saunders, Philadelphia, 1997, with permission.)



With the stylet in place, align the needle along the long axis of the humerus and perpendicular to the cortical surface of the greater tubercle.

To penetrate the cortex, apply forward pressure while rotating the needle clockwise and counterc



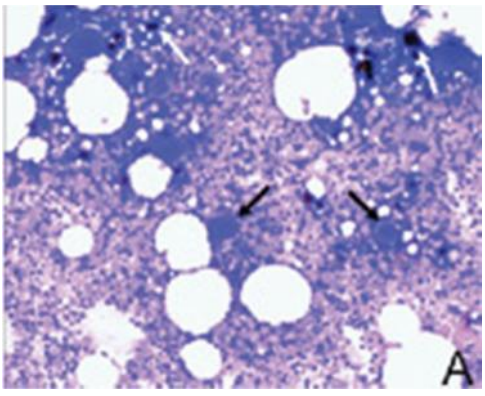


Collecting bone marrow from the sternum of a horse.

a- Express a small amount of marrow onto a slide, and tilt the slide to allow excessive blood to flow away from the spicules.

b -Place a second slide on top of the spicules perpendicular to the first slide, and gently squash the spicules as you pull the second slide across the first, and use it to gently squash the marrow particles or spicules. Gently pull apart the two slides, spreading the marrow spicules across the slides.

c- Allow the smears to dry before fixing with methyl alcohol and staining with appropriate stain.



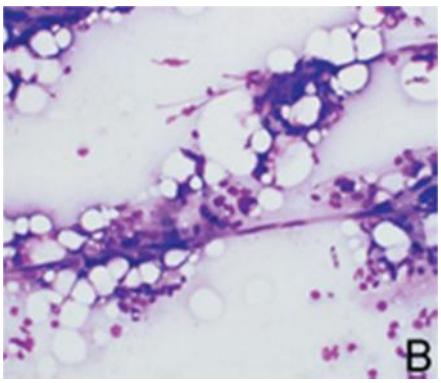
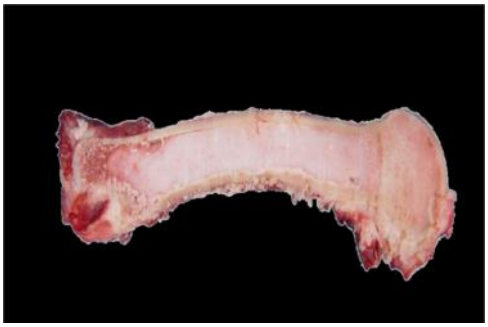


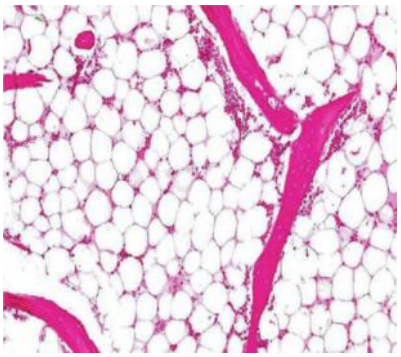
Figure A. Note the highly cellular spicule with abundant iron (white arrows), several large megakaryocytes (black arrows), a heterogeneous population of hematopoietic cells, and large, lipid vacuoles.
Fig. B-The microscopic appearance of a poorly cellular spicule, which may be secondary to poor sampling or reflect true hypocellularity.

Bone marrow aspirate/smears: Examined for:

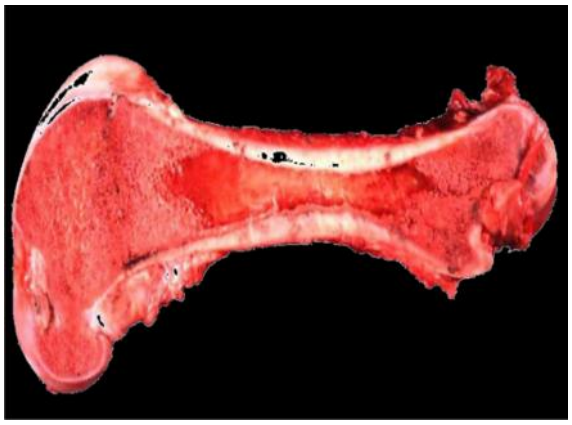
Aspirate examined

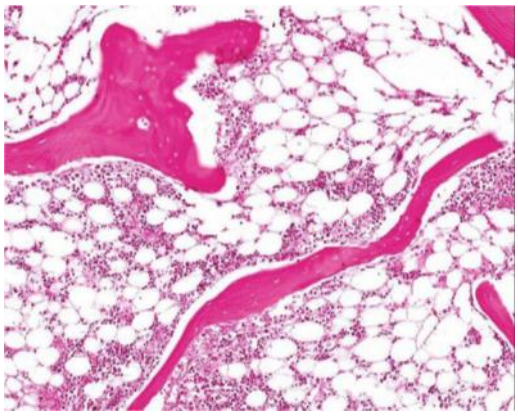
- Cellularity and Cellular morphology; Cellularity means In the bone marrow changes are reflected as increased or decreased Cellularity.
- Changes in the proportion of hematopoietic tissue (red marrow) to adipose tissue (yellow marrow), described as hypocellular, hypercellular or normal cellularity.
- Erythroid to myeloid ratio.
- Primary or metastatic neoplasia





Gross → Increased yellow marrow (Hypoplastic bone marrow)
Histo → Increased ratio of fat to hematopoietic cells





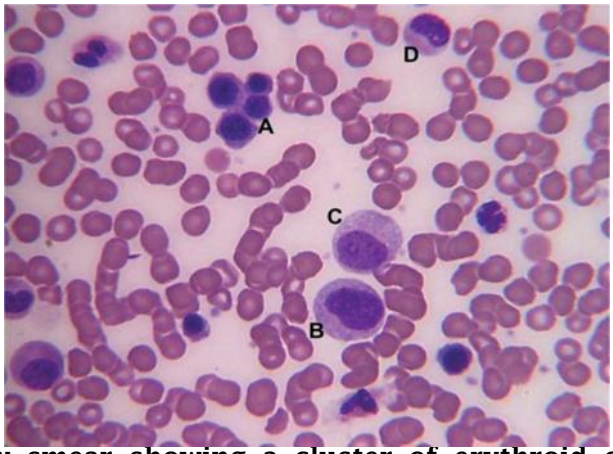
Normal bone marrow- cellularity ~ 50/50

Smears examined

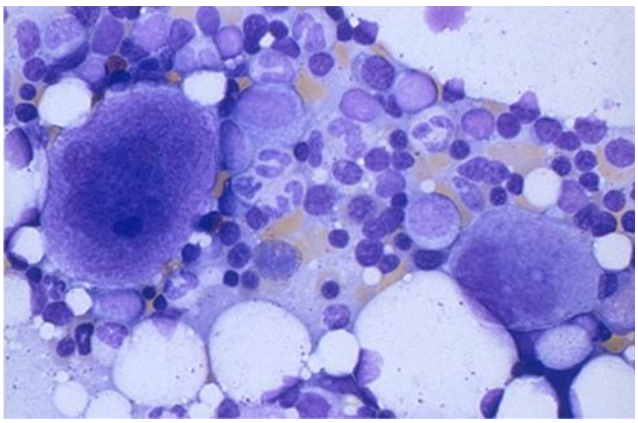
- Smears should be examined using the low power to evaluate cellularity and adequacy of megakaryocytes.
- Normal marrow appears heterogeneous; as general erythroid precursors appear smaller, have nearly spherical nuclei with more condensed chromatin, and have darker cytoplasm than do granulocytes precursors at similar maturation stage.
- A myeloid to erythroid (M: E) ratio is calculated by examining 500 cells and determining the ratio of granulocytic cells

(including mature granulocytes) to nucleated erythroid cells. Normal M: E ratio:

Dog: 0.75-2.5, cat: 1-3, horse: 0.5-1.5



Bone marrow smear showing a cluster of erythroid cells (A), neutrophilic myelocyte (B and C), and an early neutrophilic metamyelocyte (D).



Heterogenous cellularity of bone marrow- note the large megakaryocytes

Notes : An EDTA-containing solution can be prepared by adding 0.35 mL of sterile saline to a 7-mL EDTA tube, which yields a 2.5% or 3% EDTA solution, depending on whether the tube contains dry EDTA or EDTA dissolved in water. About 0.5 mL of this EDTA solution is drawn into the syringe using a regular needle prior to attaching the syringe to the bone marrow aspiration needle.

Q / If no anticoagulant is used, smears must be prepared within seconds after bone marrow collection

Q / Smears prepared once clotting begins cannot be evaluated.

Q / We prefer to collect bone marrow into a syringe that contains EDTA as an anticoagulant.

Q / Although smears need not be made immediately when collected with EDTA, they should be prepared within minutes after collection.