Bone Marrow Cytology

SAMPLE REQUIRED:
Bone marrow slides, Bone marrow aspirate in EDTA, Peripheral blood in EDTA

BLOOD TUBE REQUIRED:
EDTA tubes for bone marrow and peripheral blood

Indications:

- Investigation of unexplained cytopenias.
- Investigations or atypical cells in the peripheral blood.
- Investigation of haemic neoplasia.

Collection Protocol:

Marrow sampling:

In dogs and cats, bone marrow can be aspirated using sterile technique from the iliac crest, femur, or humerus using an appropriately sized bone marrow aspiration needle and syringe. The site aspirated will vary according to several factors including size and age of animal and degree of obesity.

Smear preparation:

Bone marrow degenerates rapidly after collection. Smears should be prepared immediately after collection. Prepare as many smears as possible with the available marrow. Smears may be sent unstained to a laboratory, or may be stained using a routine haematological stain. Because bone marrow smears are thicker than ordinary blood smears, longer staining time or double-staining is required for adequate stain quality. Leave several smears unstained for possible future use for immunophenotyping or special stains.

Smear preparation with EDTA:

A 2-3% EDTA solution (sterile) can be used to prevent clotting of the sample and to facilitate the preparation of smears. The syringe should be flushed with the EDTA solution, retaining no more than 0.1 ml per 1.0 ml of marrow. The anticoagulated sample should be placed in a plastic Petri dish. The “spicules” or “unit particles” of bone marrow may be visible as glistening fat particles suspended in blood. Tilt the Petri dish so free blood flows to the side, leaving particles visible on the bottom of the plate. Using a microhaematocrit tube, carefully pick up several marrow particles using capillary action. Transfer the particles to a clean glass microscope slide and tap the tube gently to let them flow onto the centre of the slide. Place a second clean glass slide directly over the first (longitudinally), allowing the bone marrow to spread. Gently pull the top slide off the bottom slide, lengthwise, without exerting pressure on the slide. This should result in a central, oval-shaped monolayer of bone marrow cells surrounded by peripheral blood. The central area of the smear typically is rich in unit particles.

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Bone Marrow Cytology continued...

Collection Protocol Continued...

Smear preparation without EDTA:

If EDTA/isotonic saline solution is not used, as soon as a few drops of marrow sample appear in the syringe, the plunger is released, the syringe is detached from the needle, and the stylet is replaced in the needle. The needle remains embedded in the bone. The sample is immediately expelled directly onto a glass microscope slide that is tilted at 45-70º, allowing the sample to drain from the slide into a watch glass or Petri dish. Marrow flecks/particles tend to adhere to the glass microscope slide. Smears are then prepared as above.

A peripheral blood sample should also be obtained on the same day as marrow collection. This is essential because rapid changes can occur in peripheral blood counts and accurate interpretation of cells in the marrow require knowledge of FBC results.

Notes:

If bone marrow cores are collected for histology in the same procedure, these must be shipped with a separate request form in a separate sample bag. Shipping cytology and histology samples to the laboratory in the same bag can result in exposure of the cytology samples to formalin, frequently making them unreadable.

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