Newsletter August 07

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# Bone Marrow Cytology

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**Bone marrow cytology is an important ancillary diagnostic procedure that can be used in small animal medicine.**

The **main advantages** in comparison to core biopsy (for histopathological examination) are:

- An easier technique
- An earlier availability of specimens for interpretation
- A better possibility to assess the morphology of individual cells.

The most significant **disadvantages** are:

- The restricted ability to assess bone marrow architecture, which is essential for the diagnosis of selected diseases (esp. myelofibrosis) and cellularity
- Possibly limited sensitivity to detect local alterations, such as granulomas and bone marrow metastases.

**The basic indication for performing a bone marrow evaluation is to answer questions that a routine blood film examination does not answer.** One need not take the additional effort to take a bone marrow aspirate and biopsy if for example the blood already clearly indicated there was an immune mediated haemolytic anaemia, typical inflammatory response or if even a leukaemia with clearly diagnostic features in EDTA blood.

**Indications to perform bone marrow cytology would include:**

**1. Investigation of unexplained cytopaenias**

a. Unexplained nonregenerative anaemia, especially when persistent and progressive.
b. Unexplained leukopaenia (neutropaenia), especially when a left shift is not apparent.
c. Unexplained thrombocytopaenia, to rule out decreased production as a causative mechanism.
d. Bi-cytopaenia or pancytopaenia.

**2. Investigation of atypical cells in the peripheral blood**

a. Inappropriate NRBC in peripheral blood (i.e. in the absence of a markedly responsive anaemia).
b. Immature haematopoietic cells (blasts) in the blood-atypical or immature haemic cells raise the possibility of haematopoietic neoplasia. Bone marrow evaluation is necessary to determine the source and number of these cells in the tissue.
c. Atypical RBC (e.g. basophilic stippling, multiple Howell-Jolly bodies), WBC or platelet morphology.
d. Persistent, unexplained marked increases in RBC, WBC or platelet numbers in the peripheral blood.
e. Evaluation of infectious diseases such as aspergillosis or leishmaniasis.

**3. Investigation of haemic neoplasia**

a. Differentiation, diagnosis, and staging of leukaemias and lymphomas.
b. Diagnosis and staging of other neoplasias, including histiocytic neoplasia, multiple myeloma, mast cell neoplasia and metastatic carcinoma (see fig.1).
c. Unexplained hypercalcaemia or fever of unknown origin. These may occur as paraneoplastic syndromes secondary to lymphoid neoplasia or other neoplasms affecting the bone marrow.

**Contraindications:**

On the assumption that an adequate technique is used, severe complications, including bone marrow infections, injuries of the surrounding tissue and haemorrhages, are rare. There are no absolute contraindications against the aspiration of bone marrow.

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## Bone Marrow Collection Technique For Cytological Examination

When a high-quality bone marrow aspirate is obtained, and depending on the reasons for doing the bone marrow examination, sometimes an aspirate (without a core biopsy for histopathological examination) is sufficient for evaluation. **Peripheral blood (i.e. a Full Blood Count) is ALWAYS needed for accurate interpretation of bone marrow findings.**

There are many references (listed below) in the literature explaining techniques for collecting bone marrow samples for cytological examination. Generally the following procedure may be used:

**A) Instruments and supplies required:**

- 15- to 18-gauge , 1 – 5 cm long bone marrow aspiration needle*
- Clear Petri dish or watch glass
- Several clean microscope slides
- Supplies for a surgical prep
- 5 – 10 ml syringe
- 1 ml of a 3 % EDTA/isotonic fluid solution (optional)**.

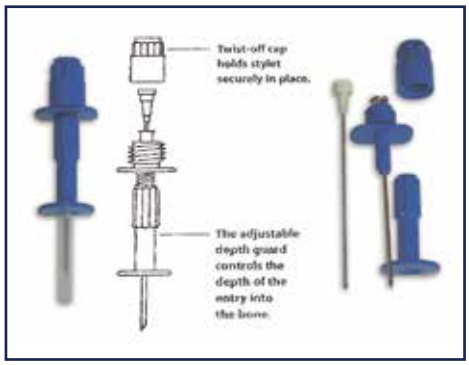


Illustration of Illinois Bone Marrow Needle

**B) Bone 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. For more information about choosing individual anatomic sites for aspiration please refer to the references below.

**C) 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 haematologic 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. If bone marrow samples are to be collected from animals to be euthanised or that have died, this should be organised within minutes of the animal's death for satisfactory preservation of cell morphology to be achieved.

**• 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.

**• 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. A second glass microscope slide is placed 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.

**D) Sending smears to the laboratory for examination:**

As for other cytological smears, bone marrow smears should be labelled and transported in sturdy slide holders and should not be packaged together with any histopathological submissions unless adequately separated. Formalin vapours adversely affect the staining reaction of cytology samples.

*cont >*



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**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.**

*** Bone marrow aspiration needles supplied by Symbion Vetnostics:**

Illinois Bone Marrow Needle 15 gauge adjustable length (1 to 5 cm ) – cost $33.00 (including GST)

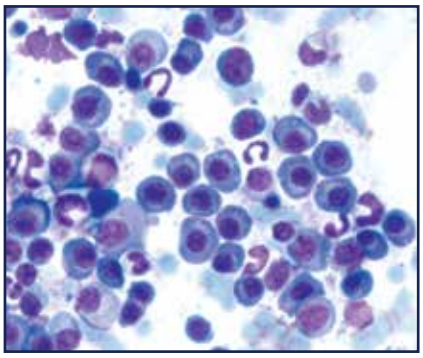

Figure 1: Bone marrow aspirate from multiple myeloma showing numerous plasma cells.

**** Recipe for making 3% EDTA/isotonic solution:**

In practice a 3% EDTA/isotonic solution can be made by using either the 4 ml or 10 ml Becton-Dickinson EDTA vacutainer blood tubes we supply as follows:

- Add 0.6 mls of isotonic saline to a 10 ml EDTA tube or
- Add 0.24 mls of isotonic saline to a 4 ml EDTA tube (you may need 2 or more of these tubes to obtain a total volume of ½ ml or greater of 3% EDTA solution)

**REFERENCES:**


1) Textbook of Veterinary Medicine 6th Edition (2005) Ettinger S.J. & Feldman E.C. Chapter 77: Techniques for bone marrow aspiration pg 285-288

2) Diagnostic Cytology of the Dog & Cat 2nd Edition (1999) Cowell R.L. et al. Chapter 23 pg 284-288

3) Atlas of Veterinary Haematology (2001) Harvey J.W. pg 93-101

4) Schalm's Veterinary Haematology 5th Edition (2000) Feldman B.f. et al Chapter 5: bone marrow evaluation pg 29-32

5) Veterinary Clinics of North America: Small animal practice (1989): Clinical pathology Part 1 Small Animals: Bone marrow biopsy and evaluation Grindem C.B. pg 669-696


## Equine Stud Season: Pre-Breeding Cultures

**by Dr Angela Begg**

It is that time of the year again when uterine and clitoral swabs are performed prior to mares going to stud. It is a requirement of some sections of the thoroughbred breeding industry that mares have a negative uterine culture prior to being bred. The requirements for clitoral cultures vary. Some studs will accept one negative culture at the beginning of the season, while others require a negative culture at the beginning of the oestrus period. It is advisable to check the requirements beforehand, as from a logistic point of view we encourage veterinarians to collect clitoral samples in batches from mares at the beginning of the stud season if at all possible to avoid delays waiting for results when the mares are ready to be bred.

Uterine swabs are collected at the beginning of oestrus (as early as possible) using guarded endometrial swabs (obtained from regular veterinary supply companies) and need to be placed in transport media prior to being sent to the laboratory. Any significant bacterial growth from uterine swabs is identified with antibiotic sensitivities performed. Beta haemolytic Streptococci are not further identified, and no sensitivity testing is undertaken as these isolates are routinely sensitive to penicillin. Clitoral swabs are cultured specifically for Klebsiella pneumoniae and Pseudomonas aeruginosa only, and are reported as negative or positive for these two bacteria only.

We perform these cultures as quickly as possible. Preliminary results of both swab types will be reported the day after receipt in the laboratory and final reports mostly issued two days after receipt. Cultures with suspect Klebsiella isolates may take another 24 hours for final identification and reporting. The lab aims to have these results out by 8.00am each day. Please remember that preliminary results may change.

It should be noted that while antimicrobial sensitivities to enrofloxacin are reported for K. pneumoniae and P. aeruginosa isolates from clitoral swabs, this antibiotic should not be used as an intrauterine or vaginal infusion. It is an irritant and will cause infertility.

It is also essential that individual swabs are labelled with the mare's name, owner, date and type of swab please.

## Vet Supply Requisition Forms

Please note that Vet Supply Requisition Forms used to order clinic supplies are now available from our website **http://www.qml.com.au/Vetnostics.asp**.

Alternatively contact our Vetnostics Department on **(07) 3121 4013**.

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## Reintroduced Tests

**Feline Heartworm Antibody, serum sample**

Cost: $40.00 + GST

**PTH related Peptide (PTH rP)**

Cost: $90.00 +GST

**PTH plus PTH rP**

Cost: $145.00 +GST

This test is available for canine, feline and equine.

Please note that this sample requires a special collection tube and processing as indicated below. This sample can also be used for the measurement of PTH.

**Time of Collection:**

Blood can be taken at any time – fasting not required.

**Collection tube:**

EDTA containing Trasylol (this is supplied on request and must be stored refrigerated until use). No other type of sample e.g. serum is acceptable.

**Minimum volume for test:**

1 ml i.e. at least 2 ml whole blood needs to be collected OR 3 ml if sample also required for PTH measurement.

**Specimen Handling:**

Chill the EDTA/Trasylol tube in a cup of ice.

Place the whole blood into the tube, invert a few times to mix.

Immediately centrifuge the tube and remove the plasma to a new tube (not one containing EDTA). If plasma cannot be separated from the whole blood immediately, it can be stored cold for up to two hours before separating.

Freeze the plasma immediately and keep frozen.

REQUEST delivery of sample in dry ice or similar to laboratory.

## Wine Winner

The winner of the 3 bottles of wine drawn from those submitting an e-mail address was Currumbin Veterinary Surgery.

For any further information please contact the Vetnostics Department at QML Pathology on **(07) 3121 4013** or visit the QML Pathology Vetnostics website **www.qml.com.au/vetnostics.asp**