

Alison Farr BVetMed MRCVS



Alison graduated from the Royal Veterinary College in London in 2000 and spent five years in small animal practice where she developed her interests in

pathology, internal medicine and exotic animal medicine before joining IDEXX Laboratories Ltd as a trainee in clinical pathology in 2005. Alison left in 2008 to have a family and returned to small animal practice for several years, before rejoining the company in June 2012 to complete her training. She has a special interest in exotic animal medicine. She has also co-written three veterinary nursing textbooks.

E-mail: alison-farr@idexx.com

**Kathleen P Freeman
DVM BS MS PhD DipECVCP
FRCPath MRCVS
RCVS Specialist in Veterinary Pathology (Clinical Pathology)
European Veterinary Specialist in Clinical Pathology**



Dr. Freeman is a Senior Clinical Pathologist for IDEXX Laboratories Ltd, Wetherby, West Yorkshire, United Kingdom. She has special interests in cytology, quality assurance and quality control, and equine pathology. She also is a consultant for Veterinary Information Network (VIN). She is a member of the European College of Veterinary Clinical Pathology Laboratory Standards Committee. She farms with her husband, Iain Kelly, in Scotland and enjoys working with sheep and Border Collies on their farm.

Cytology: common pitfalls and diagnostic dilemmas

Cytology is a frequently used and valuable tool in practice. We have compiled a short series of questions based on some common pitfalls and diagnostic dilemmas that are frequently encountered in practice. The ability to identify some of the following features can significantly improve the quality of both in-house and external laboratory cytological evaluation.

Key words: cytology, mesenchymal, round cell, epithelial

Q1: Haemorrhage – real or iatrogenic?

In assessing the presence of erythrocytes (haemodilution) in a cytological preparation, the question is whether it is peripheral blood contamination associated with sampling or truly a part of the lesion sampled. Figures 1A-C show cytological features which can indicate whether the blood has been there for a short time or somewhat longer. Identify these intracellular inclusions and the approximate duration of haemorrhage that they represent.

Answer: Figure 1A contains macrophages which have engulfed erythrocytes ('erythrophagocytosis'). If the slide was prepared immediately after sampling, this finding indicates that the bleeding occurred prior to sampling (and is not solely iatrogenic haemorrhage). If processing of a fluid sample is delayed, some in vitro erythrophagia may occur and the significance may be uncertain.

The central macrophage in Figure 1B contains blue-green to black pigment consistent with haemosiderin (these cells may be called siderophages or haemosiderophages). Haemosiderin is an iron-containing breakdown product from haemoglobin and generally indicates that haemorrhage occurred at least several days prior to sampling. A Prussian blue stain can be used to distinguish haemosiderin from bile which may have a similar appearance.

Several of the macrophages in Figure 1C contain golden rhomboid crystals consistent with haematoidin which is also a breakdown product from haemoglobin, but in contrast with haemosiderin, haematoidin does not contain iron.

There are no studies that show uniform time scales for seeing haemosiderophages and haematoidin crystals in specimens

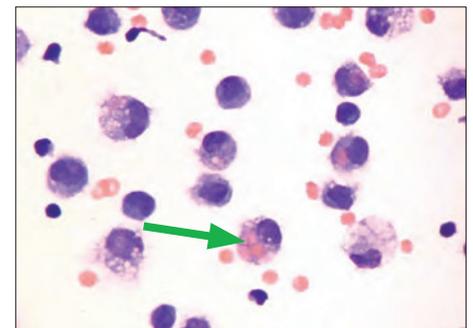


Figure 1A: Fluid-filled swelling on sagittal crest, puppy

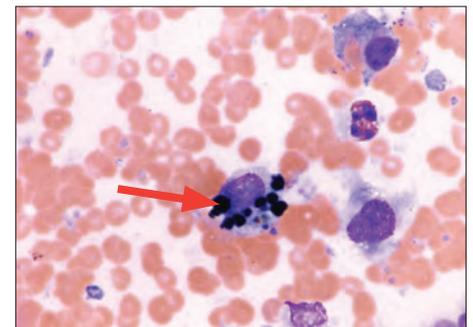


Figure 1B: Abdominal fluid, elderly mixed breed dog

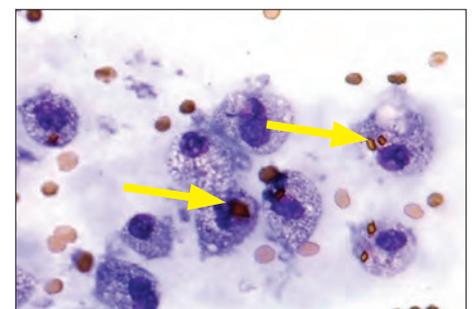


Figure 1C: Fluid-filled swelling under the chin, 4 year old Dachshund

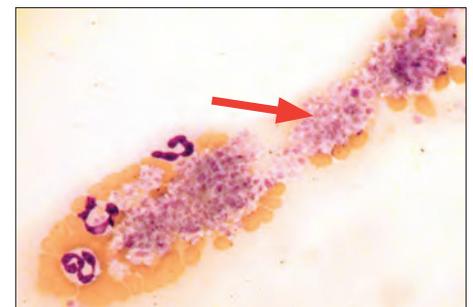


Figure 1D: Synovial fluid sample, dog



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with bleeding. In the author's experience, erythrocytes and erythrophages are expected with recent bleeding for up to 3 days and haemosiderophages and haematoidin crystals tend to occur after this time period.

The presence of platelet clumps in a sample (see Figure 1D) indicates that the haemorrhage has been very recent and is most commonly associated with iatrogenic blood contamination of the sample. Platelet clumps are typically absent by ~20-30 minutes post haemorrhage.

Q2: Acellular lesions

In most cases, a diagnosis is not possible in the absence of intact cells. However, there are occasions when the extracellular material is highly suggestive of a diagnosis. These photomicrographs illustrate two conditions that can be diagnosed in the absence of nucleated cells. Can you identify the lesions?

Answer: These photomicrographs represent aspirates from (Figure 2A) a focus of calcosinosis circumscripta and (Figure 2B) from a keratinizing lesion, such as a keratinizing cyst.

Figure 2A -The calcosinosis circumscripta aspirate contains a background of diffuse granular material with many refractile crystalline fragments of varying sizes. The number and type of refractile granules varies in aspirates. There may or may not be a few macrophages. When the slide is viewed macroscopically by 'shirt sleeve evaluation' (held with a white shirtsleeve as the background) the surface of the smear will appear 'chalky'. Usually this type of lesion is a solitary mass occurring over an extremity or pressure point, but sometimes may be bilateral or occur at multiple sites. It also may occur in the oral cavity. Surgical removal is the recommended treatment.

Figure 2B -The keratinizing lesion contains numerous squames, often in clumps and sheets, on a slight proteinaceous background. There may be associated inflammation due to leakage of cyst contents into the dermis with an associated 'foreign body' reaction, but in many patients, only the keratinaceous material is apparent. The most common causes of keratinizing lesions are follicular or epidermal cysts, but some tumours of epithelial origin may contain cysts with the same contents. Since cysts are not expected to resolve spontaneously, surgical removal is recommended, with histologic evaluation to determine the most definitive diagnosis.

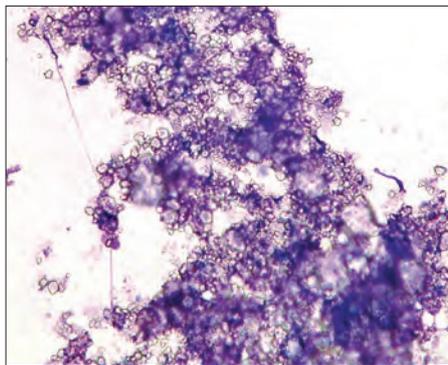


Figure 2A: Firm discrete subcutaneous mass on elbow, Rottweiler

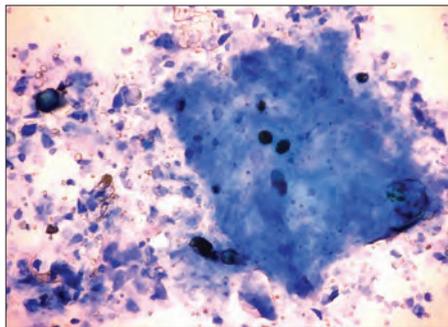


Figure 2B: Firm, cutaneous mass, Cocker Spaniel

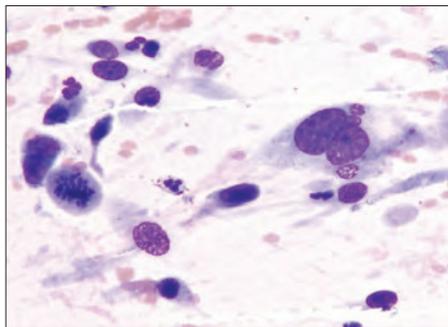


Figure 3A: Firm, poorly defined mass distal to hock, elderly cat

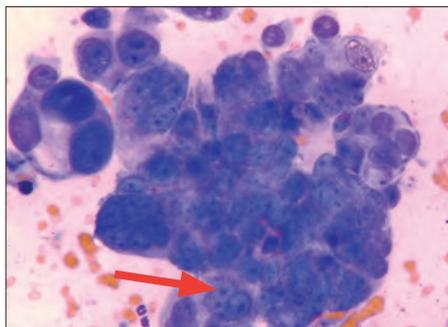


Figure 3B: Firm, poorly defined mass distal to hock, elderly cat

Q3: Malignancy

Cytological examination is a useful tool for diagnosing neoplasia, and categorisation as 'benign' or 'malignant' may be possible using various cytological features of the cell population. List the features of malignancy that may be seen in cytological specimens (some but not all features are illustrated in Figures 3A and 3B).

Answer: Some common features of malignancy include:

- Pleiomorphism – variation in shape and size, often with increased cell size
- Increased nuclear:cytoplasmic ratio
- Anisokaryosis (variation in nuclear size) and multinucleation
- Nuclear molding (nucleus from one cell wrapping around or molding the contours an adjacent nucleus)
- Prominent nucleoli, multiple nucleoli and anisonucleoliosis (variation in size of nucleoli); the red arrow shows these features as well as nuclear molding
- Increased numbers of mitotic figures, and abnormal mitotic figures

It is important to note that some of these features can be seen in benign proliferations, for example the mesenchymal cells in reactive fibroplasia can exhibit significant pleiomorphism, and epithelial cells may show dysplastic features (e.g. increased nuclear to cytoplasmic ratio, increased cytoplasmic basophilia, increased anisocytosis and anisokaryosis, increased numbers of immature cells, asynchronous maturation of the nucleus and cytoplasm) in response to inflammation. Some normal tissues such as lymphoid tissue show a variation in maturity of the cells and in cell size. Additionally, some malignant neoplasms such as thyroid adenocarcinomas exhibit few cytological criteria of malignancy despite malignant biological behaviour. So, evaluation of these features must occur within the context of the type of lesion, clinical history, presentation and results of other evaluations in order to determine if malignancy is likely.

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1. Excessively blue staining slides with densely stained nucleated cells and poor cellular contrast can be the result of:

- A) Exposure to formalin fumes
- B) Excessive ultrasound jelly
- C) Delayed preparation of smears
- D) Poor microscopic light adjustment
- E) Poor cellular fixation

Questions continue on page 13!