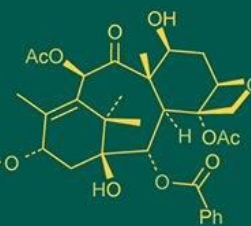
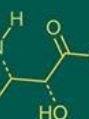
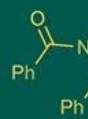


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Diagnostic cytology: Key techniques and applications in disease diagnosis

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Abstract

Diagnostic cytology involves the examination of individual cell morphology for the purpose of diagnosing and predicting the progression of diseases. It is primarily used because cytological samples are more affordable, easier to collect, simpler to process, and facilitate a rapid diagnosis. Techniques employed in diagnostic cytology include smear preparations (e.g., blood or impression smears), fine-needle aspiration, which incorporates methods such as the woodpecker technique, syringe-attached method, and squash-preparation. Additional techniques include tissue scraping, swabbing, and biopsy. Recognizing basic inflammatory cells, such as neutrophils, eosinophils, lymphocytes, macrophages, and plasma cells, is crucial for the accurate interpretation of cytological samples. For diagnosing neoplastic growth, cytology aids in evaluating the nucleus and cytoplasmic characteristics of the cells. Vaginal cytology is widely used to identify different stages of the estrous cycle in canines. Furthermore, analyzing body fluids, such as synovial and peritoneal fluids, contributes to diagnosing specific conditions through cytological evaluation.

Keywords: Cytology, inflammatory cells, neoplasia, diagnosis

Introduction

Diagnostic cytology focuses on analyzing individual cell morphology for diagnosing and predicting disease outcomes. It is a rapid, cost-effective, minimally invasive, and easily repeatable method that does not require specialized infrastructure. Cells and sample contents are microscopically examined, allowing for immediate on-site diagnosis. In addition to descriptive articles and reviews, numerous guides and atlases have emerged, reflecting the growing interest in this diagnostic technique, particularly for identifying infectious agents and assessing skin lesions, lymph node enlargements, and body cavity effusions (Raskin & Meyer, 2001) ^[20]. In veterinary medicine, the adoption of cytology as a diagnostic tool is relatively recent, gaining significant traction over the past two decades (Sorenmo, 2003) ^[23].

Approaches for Diagnostic Cytology

1. Smear

A. Blood smear technique

This preparation technique is commonly used for fluid samples. Even in the case of highly fluid aspirates, a squash-preparation often yields a well-prepared slide. To begin, a drop of the sample is placed on one end of a glass slide. Another slide, referred to as the spreader slide, is then drawn back into the drop. As the spreader slide contacts the sample, the fluid quickly disperses along the meeting point of the two slides. The spreader slide is then moved forward smoothly and swiftly while maintaining contact with the first slide, until a feathered edge is formed. It is crucial to ensure that the sample does not run off the slide, as this could cause larger, and often more critical, cells to be lost at the smear's edge.

B. Impression Smear

Impression smears are frequently employed for the rapid assessment of tissues collected during surgery or necropsy. In this technique, the tissue is gently rolled or dabbed onto a glass slide before being placed in formalin. Impression smears can also be obtained from exudative or ulcerated lesions. In such cases, it is crucial to manually remove any dry scabs or crusts before making the smear, as the cells in these materials are diagnostically

uninformative. For high-quality impression smears from surgical or necropsy tissue, the sample should first be cut to expose a fresh surface, followed by blotting to remove excess fluid and blood. This step is important for enhancing the smear's clarity. After blotting, the tissue is gently rolled or dabbed onto a clean glass slide, which can be repeated several times to create multiple impressions on the same slide. It is important not to rub the tissue on the slide, as this can lead to cell lysis, nuclear streaming, and ultimately, a "non-diagnostic sample."

To reduce the risk of microbial or cellular contamination, the lesion area should be cleaned using a saline-moistened cotton swab. Water should be avoided, as it can cause osmosis-induced cell rupture. When obtaining imprints from excised tissue during surgery, the surface should be freshly cut to enhance the quality of the smear.

Impression smears made from exudative or ulcerated lesions prior to cleaning typically show only inflammatory cells and organisms. However, when the lesion is cleaned with a saline-moistened gauze or surgical sponge, the smear may contain cells that more accurately represent the primary lesion, provided it involves superficial tissues. In cases where the primary lesion involves deeper tissues, such as neoplasms that have become ulcerated and infected, the smear may still yield only superficial inflammatory cells even after cleaning. Thus, this method may have limitations in cytologic interpretation if the lesion is not confined to superficial layers.

Table 1: Common Problems and Solutions for Blood smear Technique

Common Problems	Solutions
1. Poorly prepared slides	- Smear should be made immediately after expel onto slides to prevent drying or clotting - Smear should be rapidly air dried after preparation. (waving the slides or hair dryer)
2. Staining problems	- Use new slide, fresh buffer solution - Stain immediately after air drying - Don't touch the surface of the slide or smear

2. Fine-Needle Aspiratin Cytology (FNAC)

Fine-needle aspiration (FNA) or fine-needle biopsy is an excellent method for collecting cytological samples from cutaneous and subcutaneous masses, offering a minimally invasive way to sample internal organs, lymph nodes, or masses. The procedure typically involves using a 10-20 ml disposable syringe fitted with a 21-25 gauge needle. For softer tissues, smaller needles and syringes are used, while firmer tissues, such as fibroadenomas, require larger syringes to generate sufficient pressure (Meinkoth & Cowell, 2002) ^[15]. Using needles larger than 22 gauge may result in the collection of tissue cores, yielding fewer free cells for cytologic analysis.

Methods for FNAC

A. Woodpecker Technique: In this method, the needle (without an attached syringe) is inserted into the mass and redirected multiple times to collect exfoliated cells within the needle. Afterward, a 3-10 cc syringe filled with air is attached to the needle to expel the sample onto a slide. The sample is then processed using the squash-prep technique (Frable, 1989) ^[7].

B. Syringe-Attached Method: If the woodpecker technique does not yield enough cells, the syringe-attached method provides a more aggressive approach. However, this technique often results in increased blood contamination, which can interfere with cytological analysis. In this method, the needle (with a syringe attached) is inserted into the mass, and negative pressure is applied by withdrawing the plunger. While maintaining this pressure, the needle is redirected several times within the mass. Before removing the needle, the negative pressure is released to prevent cell loss deep within the syringe. Afterward, air is drawn into the syringe, and the sample is "blown-out" onto a slide for preparation using the squash-prep or blood smear technique.

Squash-Prep (Slide-over-Slide Preparation)

To perform a squash-prep, the aspirated sample is expelled onto one end of a glass slide. Another slide is gently placed over the sample, and without applying extra pressure, the slides are smoothly slid apart. Given that cells are fragile, care must be taken to avoid causing damage by applying too much force or pulling the slides apart vertically. This method ensures minimal cell trauma while providing a clear sample for examination.

2. Fine-Needle Aspiration Cytology (FNAC)

Fine-needle aspiration (FNA) or fine-needle biopsy is a highly effective method for collecting cytological samples from cutaneous and subcutaneous masses. It is minimally invasive and allows for sampling of internal organs, lymph nodes, or other masses. Typically, the procedure involves using a 10-20 ml disposable syringe fitted with a 21-25 gauge needle. Softer tissues require smaller needles and syringes, whereas firmer tissues, such as fibroadenomas, necessitate larger syringes to maintain adequate pressure during sample collection (Meinkoth & Cowell, 2002) ^[15]. Larger needles, such as those exceeding 22 gauge, tend to aspirate tissue cores, resulting in a lower yield of free cells for cytological analysis.

Methods for FNAC

A. Woodpecker Technique: In this technique, the needle (without a syringe attached) is inserted into the mass and redirected several times to collect exfoliated cells. Once this is done, a 3-10 cc syringe filled with air is attached to the needle, and the sample is "blown-out" onto a slide. The sample is then prepared for analysis using the squash-prep technique (Frable, 1989) ^[7].

B. Syringe-Attached Method: If the woodpecker technique fails to collect an adequate number of cells, the syringe-attached method is a more aggressive alternative. However, this method can result in increased blood contamination, which can complicate cytological evaluation. For this method, the needle (with an attached syringe) is inserted into the mass, and the plunger is withdrawn to create negative pressure. While maintaining this pressure, the needle is redirected several times within the mass. The negative pressure is released before removing the needle to prevent cells from being trapped inside the syringe. Afterward, air is drawn into the syringe, and the sample is expelled ("blown-out") onto a slide for further preparation using the squash-prep or blood smear technique.

Squash-Prep (Slide-over-Slide Preparation):

The squash-prep technique is performed by expelling the aspirated sample onto one end of a glass slide. Another slide is gently placed over the sample, and without applying extra pressure, the slides are smoothly slid apart. Since cells are delicate, care must be taken to avoid excess trauma by applying too much force or separating the slides vertically. This method minimizes cell damage and ensures the preparation of a clear sample for cytological examination.

Precautions during Slide Preparation for Cytology

Slide preparation for cytological examination should begin immediately after specimen collection to prevent the sample from clotting or drying out before the smear is made. A few important precautions include:

1. **Use of Needle Tip:** Always use the tip of the needle to spread the sample gently. This helps prevent distortion of the cellular structure during the spread.
2. **Avoid Thick Smears:** Control the amount of sample expelled from the syringe to avoid making smears that are too thick. Thick smears can lead to an overrepresentation of blood or other cells, obscuring the primary cell type that needs to be examined.
3. **Squash Smears:** When making squash smears, pull the slides apart gently. Excessive pressure can cause cellular disruption and make the sample non-diagnostic.
4. **Multiple Smears:** It is advisable to prepare at least four to five smears from a single specimen. This practice improves the sensitivity and specificity of the diagnosis.
5. **Use of Frosted or Colored Edged Slides:** Frosted or colored slides are preferable because they allow permanent labeling, which helps identify the correct side of the slide. This minimizes the risk of wiping off the sample during staining due to the usually colorless appearance of cytological samples.
6. **Fluid Samples:** When examining fluids cytologically,

smears should be prepared immediately after collection. This ensures better-quality samples since the cellular quality in EDTA-treated vials is generally lower than in freshly collected samples.

Cytological Interpretation

Cytological interpretation differs from classical histopathology, where tissue architecture is a primary focus. In cytology, individual cells are examined at a sub-cellular level to provide diagnostic information. The following observations are typically made in cytological samples and are reported in a preferred order:

1. **Inflammatory Lesions:** Inflammatory lesions are categorized based on the predominant cell type:
 - **Neutrophilic (Suppurative, Purulent, Acute; Septic or Non-Septic):** Characterized by a predominance of neutrophils.
 - **Mixed Cellular (PMNs and Macrophages; Pyogranulomatous):** Contain both neutrophils and macrophages.
 - **Predominantly Mononuclear (Chronic):** These lesions consist mainly of macrophages.
 - **Granulomatous Inflammation:** Involves multinucleate giant cells.
2. **Allergic Responses:** These may contain a significant number of eosinophils.
3. **Foreign Organism Search:** A thorough search for foreign organisms should be conducted in most inflammatory lesions.
4. **Bacterial Infections:** These are suspected when polymorphonuclear neutrophils (PMNs) make up more than 85% of the sample, particularly if the PMNs appear degenerate.
5. **Fungal, Chronic, or Foreign Body Infections:** These may be indicated when macrophages are present at levels exceeding 15%.

Table 2: Diagnostic Cytology of Inflammation

Neutrophilic inflammation:	Cellular Details
1. Septic (bacterial):	1. PMNs predominate with > 85% neutrophils (Tyler and Cowell, 1989) ^[26] . Degenerated nuclear change (karyolysis, nuclear swelling) of PMNs. Bacteria or other organisms seen intra- and extra-cellular. Proteinaceous background. 2. Marked toxic process: karyolysis, karyorrhexis, foamy Basophilic cytoplasm, toxic granulation, (Döhle bodies). 3. Non- toxic process: (slow cell death) karyorrhexis, pyknosis, hypersegmentation, cytoplasmic changes not much pronounced.
Nonseptic:	Mixed cellular response with > 70% non-degenerated neutrophils Immune-mediated process: PMNs (± lymphocytes and plasma cells) Eosinophilic: PMNs plus eosinophils (> 10%), plasma cells, lymphocytes. Eosinophilic inflammation can occur in allergies, hypersensitivities, some neoplasms, mast cell tumour, lymphomas, migrating parasitisms, etc.
Mixed cell inflammation:	Subacute inflammation: 50% or more neutrophils 30-50% mononuclear cells: monocytes, active macrophages, some lymphocytes, occasional plasma cells & mast cells. Chronic :> 50% mononuclear cells. Active macrophages predominate. Viral inflammation: Often a predominance of mononuclear cells, with lymphocytes and plasma cells Granulomatous inflammation: Epithelioid cells, giant cells, active macrophages & PMNs. Foreign material e.g. crystals, plant material or specific organisms.

Cytology of Inflammation

The recognition of fundamental inflammatory cells, including neutrophils, eosinophils, lymphocytes, macrophages, and plasma cells, is crucial for the accurate interpretation of cytology samples (Villiers & Dunn, 1998) ^[27]. While some tumors may contain numerous

inflammatory cells, this is rare, even in cases of tumor necrosis. The presence of inflammatory cells alone typically indicates a primary inflammatory lesion. In instances of ulceration, inflammation occurs on the surface of neoplastic lesions, but the inflammatory cells generally do not penetrate deeply into the underlying neoplasm. A small

proportion of mast cell tumors, however, may consist almost entirely of inflammatory cells, hemorrhage, and edema, with only a few mast cells. These tumors can be difficult to identify, even through histological examination.

Diagnostic Cytology of Neoplasia

Morphology of Normal and Neoplastic Cells

When analyzing sub-cellular details, several fundamental principles guide cytological interpretation for neoplasia:

A. Nucleus: The nucleus reflects the cell's growth activity, whether euplastic, proplastic, retroplastic, or neoplastic.

B. Cytoplasm: The cytoplasm indicates the cell's lineage and functional differentiation (e.g., chondrocyte, mast cell, fibrocyte) (Griffiths *et al.*, 1984) [18].

The stages of growth activity that can be identified morphologically through nuclear features include:

- 1. Euplasia:** Cells with normal growth activity exhibit regular, smooth cytoplasmic and nuclear margins and a bland, even chromatin pattern.
- 2. Proplasia:** Cells experiencing increased growth activity, often in response to hormonal stimulation, injury, or repair, demonstrate a slightly higher nuclear-to-cytoplasmic ratio, a visible nucleolus, and denser chromatin.
- 3. Retroplasia:** Cells with reduced growth activity, which may occur due to injury, aging, or starvation, have pyknotic nuclei and more basophilic cytoplasm.
- 4. Dyskaryosis:** A dyskaryotic cell has an abnormal nucleus, potentially indicating pre-neoplastic changes or toxic insults. These cells are difficult to distinguish definitively from neoplastic cells.

5. Dysplasia: Dysplasia refers to any abnormal cell development, leading to cells that differ in size, shape, and appearance (Koss, 2006) [13].

6. Neoplasia: Cells undergoing neoplastic transformation exhibit distinct nuclear and nucleolar irregularities, with significant variation in the size, shape, and number of nucleoli (Barton, 1987) [3]. Dense chromatin, indicative of increased ploidy, and a high nuclear-to-cytoplasmic ratio are typically observed. These features can vary depending on the type and malignancy of the neoplasm and whether it is epithelial or mesenchymal in origin.

As cells progress through the cell cycle, nuclear features and chromatin patterns change. During the resting (G0) phase, nuclei display a fine, sieve-like chromatin pattern. During rapid growth (prophase), the chromatin becomes stippled, and a nucleolus is present, while in the pre-mitotic phase, the chromatin becomes coarse and clumped. Malignant cells often exhibit darkened chromatin due to polyploidy, and nucleoli may become more prominent and variable. Many malignant cells are found in the G0 phase.

When diagnosing neoplasia, several cytological characteristics of malignant cells must be considered, with monomorphism of cell type being a common feature. Mitotic figures, while often noted, are not necessarily diagnostic unless the mitoses themselves are abnormal or occur in large numbers. Reporting the frequency of mitoses is also standard practice.

Cytoplasmic features are useful in identifying the lineage of neoplastic cells. However, poorly differentiated cells present greater challenges. In such cases, immunophenotyping using immunocytochemistry is recommended to detect antigenic markers located in the cytosol or cytoplasmic membrane, providing further diagnostic clarity.

Table 3: Diagnostic cytology of inflammation and neoplasia.

Observations	Inflammation	Neoplasia
1. Cell Populations (%)	Segmented neutrophils, Macrophages (Eosinophils, Plasma cells)	Monomorphic
2. Cell Morphology	Inflammatory cells: Well-preserved or degenerate (karyolysis, pyknosis, cytoplasmic basophilia)	Nuclear criteria of malignancy
3. Background	Protein, mucin, bacteria, fungi cholesterol crystals.	Cytoplasmic products

Non-Inflammatory and Non-Neoplastic Lesions

This category includes cysts, transudates, and hyperplastic lesions. These lesions generally consist of a simple accumulation of resident normal cells. For example, epidermal cysts predominantly contain nucleate epithelial squames and cholesterol crystals, although they may occasionally have an inflammatory infiltrate. In contrast, salivary cysts typically include mucinous aggregates, some phagocytes, and clusters of salivary epithelial cells (Author, Year).

Vaginal Cytology

Vaginal cytology can be used to identify the various stages of the canine estrous cycle; however, the results must be interpreted in conjunction with the animal's behavior (Author, Year). Parabasal cells are characterized by their small size, central round nuclei, indistinct nucleoli, a relatively narrow band of cytoplasm, and a nucleus-to-cytoplasm ratio of 1:1. Small intermediate cells have a similar nucleus but possess a much larger amount of cytoplasm. Large intermediate cells also have a similar nucleus but exhibit a significantly larger amount of

cytoplasm and an angular, irregular outline. Superficial cells also feature a large amount of cytoplasm, but their nuclei are either pyknotic (small and contracted) or absent.

The stages of the estrous cycle change gradually. If a preparation does not precisely match a specific part of the cycle, judgment must be used to determine which stages are present. During proestrus, all types of epithelial cells are present, along with neutrophils, red blood cells (RBCs), and mucus. As proestrus progresses, the epithelial cells increasingly approach terminal differentiation, characterized by a rise in superficial cells and a decrease in neutrophils. Bacteria are often present in large numbers. In estrus, more than 90% of epithelial cells are superficial, with no background mucus. There are large numbers of bacteria, but no neutrophils. In diestrus, parabasal and intermediate cells account for over 80% of the total epithelial cells. The presence of neutrophils and bacteria is variable but usually less than in proestrus. Distinguishing between some stages of proestrus and diestrus can be challenging. In anestrus, parabasal and intermediate cells are predominant, and neutrophils and bacteria are rare. The presence of RBCs is not particularly useful for differentiating the stages of estrus.

Body Cavity Fluid Cytology

Synovial Fluid Cytology

Direct smears of synovial fluid generally show only 1-2 cells per x400 magnification. The background is granular and highly proteinaceous, containing glycosaminoglycans.

The predominant cells in synovial fluid are small mononuclear cells, such as synoviocytes and small lymphocytes, with neutrophils making up less than 5% of the cell population.

Table 4: Synovial fluid Cytology Details.

Diagnosis	Cells Observed
Infectious arthritis	Neutrophils, bacteria, fungi or foreign organisms
Non-infections inflammation	Neutrophils
Degenerative joint disease	Mononuclear cells, < $5 \times 10^9/L$ (range 1 – 10), $\pm$ cartilage fragments > 10% large vacuolated macrophages Multinucleate or binucleate cells (Brannan and Jerrard, 2006).
Trauma	Some inflammatory cells, Usually RBCs
Haemarthrosis	Many RBCs, inflammatory cells, haemosiderophages, haematoidin crystals

Effusions

Effusions are characterized by the accumulation of excessive fluid in the pleural, peritoneal, or pericardial spaces, indicating edema within these body cavities. To

analyze these fluids, samples are collected into EDTA tubes for cytological examination. Direct and sediment smears are prepared from these samples for cytological evaluation

Table 5: Measurable Characteristics of Effusions (Baker and Lumsden, 2000) ^[2].

Determination	Transudate	Modified transudate	Exudate
RBCs	None to few	Variable	Variable
Nucleated cell count	< $0.5 \times 10^9/L$	$\geq 3 \times 10^9/L$	> $3 \times 10^9/L$
Dominant cell type	Few	Macrophages (+ others)	PMNs

3. Peritoneal fluid

Normal nucleated cell count varies with the species & site as below:

Dog & Cat : < $3 \times 10^9/L$ (often < $1 \times 10^9/L$)

Horses: $9.0 \times 10^9/L$ (often < $5 \times 10^9/L$)

Cattle: $6.0 \times 10^9/L$ (variable, increased post -partum)

Limitations of Cytology

Cytopathology as an Adjunct to Clinical Diagnosis

Cytopathology serves as an adjunct to clinical diagnosis, providing rapid and reliable results when performed with skill. However, it does not replace histopathology. In some instances, it may only offer a differentiation between inflammation and neoplasia, while in other cases, a more definitive diagnosis can be made depending on the experience of the cytologist interpreting the slides (DeMay, 1996) ^[5]. It is advisable to examine both cytology and histology preparations in parallel when starting this study. Proper collection and handling of samples are crucial, as improper techniques may result in misleading information. While a positive cytological diagnosis of malignancy is significant, a negative finding does not necessarily rule out malignancy. Additional or alternative tests may be required. Nevertheless, the initial cost of cytological examination is minimal, and the sample collection requirements are relatively simple (DeMay, 1996) ^[5].

Writing Microscopic Descriptions in Cytopathology

When writing microscopic descriptions, it is essential to clearly differentiate between observations and interpretations. For example, describe a large mixed population of bacteria as normal flora or identify dark blue-black granular pigment consistent with hemosiderin. Descriptions should be written in the present tense using an active voice and should be concise. Avoid redundancy,

indicate what “is observed” rather than what “is present,” and minimize the use of ambiguous terms such as “appear to be” and “generally.” Avoid subjective terms like “very,” and ensure subject-verb agreement (e.g., a large number of neutrophils is observed). The cytologic diagnosis should be a brief summative interpretation of the description and may include an indication of the level of confidence in the diagnosis (DeMay, 1996) ^[5]. Comments may be used to discuss differential diagnoses and their relative likelihood, address specific clinician concerns (e.g., no evidence of metastasis), recommend follow-up tests (e.g., culture and sensitivity, histopathology), and provide other decision support information. In summary, concise, complete, and well-organized microscopic descriptions effectively communicate cytologic findings and improve the quality and value of cytology reports (DeMay, 1996) ^[5].

Conclusion and Future Prospectus Veterinary Cytopathology

Cytology is a highly useful diagnostic method in veterinary pathology. It is quick, inexpensive, and accurate, with minimal risk to animals during sample collection. Effective cytological practice requires good communication with clinicians and correlation with other diagnostic methods such as histopathology. Veterinary diagnostic cytopathology demands continual learning and education (Jha *et al.*, 2013) ^[12].

Image-Guided FNA Cytology

Image-guided fine needle aspiration (FNA) cytology, using techniques such as ultrasound or radio waves for guidance, is a simple, safe, and cost-effective screening test for deep-seated lesions and for evaluating internal organs (Jha *et al.*, 2013) ^[12].

Telecytology

Telecytology involves scanning, capturing images, and performing online diagnosis, which facilitates remote consultations and diagnostics (Hebert *et al.*, 2001) ^[11].

Use of Additional Stains

Additional staining techniques, including immunocytochemistry, are employed to detect the presence of specific proteins in cell suspensions and cultured cells using targeted antibodies.

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