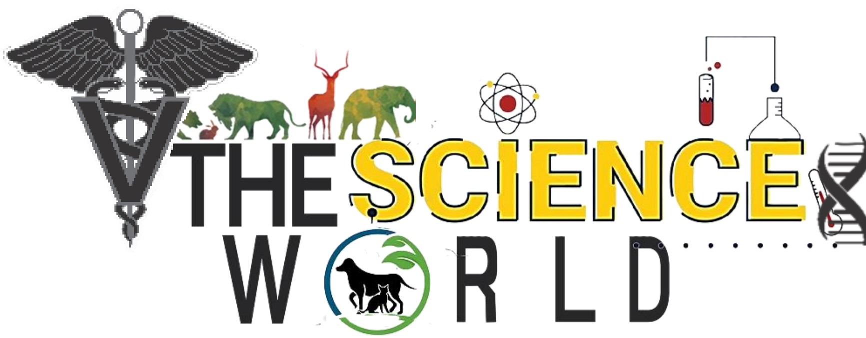

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Popular Article

Diagnostic Cytology in Veterinary Medicine

Sarjna Meena, Anita Rathore, Samita Saini, Manju, Sandeep Marodia, Sunil Jangid and Priya Saini
Department of Veterinary Pathology,Post Graduate Institute of Veterinary Education and Research, Jaipur (RAJUVAS)-302031
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Phylogenetically ranging from invertebrates to marine mammals, cytology is a core diagnostic pathology service that uses specimens from domestic animals, laboratory animals, and foreign species. Cytology was first used to identify cancer and potential pre- cancers affections by George Nicholas Papanicolaou in 1928. The science of cells known as cytology, is used to distinguish between healthy, malignant and inflammatory cells. It is more widely accepted than other diagnostic techniques since it is quick, affordable, minimally intrusive and simple. It includes methods like centesis, fine needle aspiration, impression, scraping, swabs and catheterization for sample collection. Furthermore, squeezing, blood smearing, needle spreading and line smearing procedures are used in the preparation of slides. Cytology is so special and unique in that it can provide disease outcomes while the patient is still with us. This science is also widely used to diagnose both internal and external illnesses, including neoplastic alterations. In the case of neoplastic diseases, it is important to differentiate between neoplastic nuclei, neoplastic cytoplasm and neoplastic structures of different cells with their normal cells, as these features are abnormal in neoplastic diseases. A basic evaluation of the major pathologic process or processes such as inflammation, the presence or absence of infectious agents, hyperplasia, benign and malignant neoplasia, is made in this field after samples have been screened for quality. In many cases, depending on the sample's quality and the lesion's characteristics, a more precise diagnosis can be made (e.g., determining the etiologic agent or classifying a particular neoplasm) but this is not always the situation, in these cases, additional

testing (typically a biopsy with histopathology, molecular studies, or infectious agent culture) will be advised.

Branches of Cytology

Aspiration cytology

Several names are used to refer to this expanding method. The most well-known ones are FNA, specifically, Fine Needle Aspiration Cytology (FNAC), Fine Needle Aspiration Biopsy (FNAB) and Needle Aspiration Biopsy Cytology (NABC). These all refer to the same process of utilising a small needle to aspirate cellular matter in order to make a diagnosis. This method has been applied to both palpable and non-palpable lesions, two primary categories of body lesions.

Exfoliative cytology (body cavity effusion cytology)

The samples are made up of cells that exfoliate from superficial or deep mucosal or serosal surfaces. High numbers of immature (blast) cells such as lymphoblasts, can be found within a neoplastic effusion or as can epithelial cells (rarely spindle cells) that meet the necessary requirements for malignancy to be diagnosed as neoplasia.

Basic Cell Types

1. Non-inflammatory cells

According to specific cytological characteristics, cells can generally be divided into three groups, discrete cells (commonly known as "Round cells"), epithelial cells and mesenchymal cells. The evaluator can more easily determine the particular cell type present by classifying the cells into the primary group to which they belong. Even if a precise identification cannot be achieved, significant information can be learned, such as whether a cell type that is atypical for the tissue examined is present (e.g., epithelial cells in a lymph node aspirate).

Discrete cells (round cells)

Because they exist separately in tissues and are not adhered to other cells or the connective tissue matrix, thus discrete cells are a group of cells that share specific cytological characteristics. They are primarily hematogenous origin mobile cells. Normal lymphoid tissue aspirates, like those of the spleen and lymph nodes, produce cell populations with this distinct cell pattern. However, the majority of discrete cell cancers exhibit cytological features that are distinctive enough to enable a particular diagnosis. Mast cell tumour, lymphosarcoma (lymphoma), histiocytoma, plasmacytoma and transmissible venereal tumour (TVT) are the distinct cell cancers. Moreover, melanomas are a

master of mimicry, producing cell populations that can resemble distinct, epithelial or mesenchymal tissue.

Epithelial cells

Many cytological preparations frequently contain normal epithelial cells. The majority of surface scrapings and swabs contain surface epithelium, including columnar cells from transtracheal washes and squamous cells from skin scrapings, nasal or vaginal swabs and as the result of normal exfoliation (transitional cells from urine sediments). Neoplasms or hyperplasic proliferation can also give rise to epithelial cells.

Mesenchymal cells

The genesis of mesenchymal cells is connective tissue. Hematopoietic cells, which contain many of the cells referred to as "Discrete cells" are included in mesenchymal tissue because blood is a connective tissue. These hematopoietic cells are often categorised independently since their cytological appearance is so different from that of the other connective tissues. The term "Mesenchymal cells" is frequently used in cytology texts to refer to "Stromal" connective tissue cells.

2. Inflammatory cells

Neutrophils, macrophages, lymphocytes, and eosinophils are the four primary types of inflammatory cells. Because most clinicians have seen inflammatory cells in peripheral blood smears several times, they are more accustomed to their morphology and initially feel more confident identifying them.

Types of Cytological Techniques

Solid tissue sample collection techniques

Depending on the form and location of the lesion, the differential diagnosis list and the patient's disposition, the best sample techniques for cytology may change. Swabs, impression smears, scrapings, tape preparations, and needle aspirates are some sample types that can be used for cytological analysis. These different kinds of samples are obtained by using the various techniques described below

1. **Fine needle aspiration techniques**

The most effective and widely used technique for sampling proliferative masses and neoplastic lesions. As a general rule, the softer the tissue, the smaller the needle and syringe required to collect an acceptable sample from organs like the liver or spleen. Often recommend, using a 22–25-gauge needle and a 2-5 ml syringe for this technique. It is a helpful diagnostic tool for examining soft tissue masses including cutaneous lesions, lymph nodes and intra-thoracic or intra-abdominal masses and effusions from body cavities. The manoeuvre is simple to execute in a practise environment. An "Aspiration technique" or a "non-aspiration technique" can be used to take fine-needle aspirates.

Aspiration technique

Once the mass is stabilized between the operator's fingers, the fine gauge needle is inserted into the mass. When the needle is seated comfortably in the mass, negative pressure is applied to the plunger/syringe. Try and avoid redirecting the needle or moving it back and forth within the mass whilst vacuum (negative pressure) is applied as this generally results in increased blood contamination of samples. This procedure should be repeated at least 3–4 times at different angles within the lesion to obtain a representative cell population from the lesion in question. Smaller syringes attached to the needle offer the operator better control during the aspiration process, particularly when aspirating smaller lesions. A minimal amount of material within the hub of the needle is adequate and generally this is sufficient for cytological interpretation. Attempted further aspiration often leads to unwanted blood contamination. If blood is encountered during aspiration attempts, then the exercise should be ceased and repeated a little further away from the initial puncture site. Negative pressure should be released before the needle is removed from the mass and skin. Once the needle is removed from the syringe, air is drawn into the syringe and the needle is firmly reattached to the syringe.

Non aspiration technique (woodpecker method)

The non-aspiration technique is preferred for sampling of all masses or organs which are highly vascular (e.g., spleen, liver) in order to minimize blood contamination. Overall, the sampling procedure should take no longer than 5-10 s and several smears should be prepared. The non aspiration technique using a 'needle alone technique' is useful for obtaining samples from small lesions such as pustules. The non-aspiration technique with the syringe attached to the needle is used

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here to sample the spleen of a dog with ascites and icterus under ultrasonographic control. Note the syringe is prefilled with air and is held between the thumb and forefinger.

2. Impression techniques

Skin ulcers, exudative lesions, surgically excised masses, and tissues from necropsies can all yield impression smear samples. It is important to imprint, clean, and reimprint ulcers. The tissue to be imprinted should first be cut in half to have a fresh surface in order to acquire impression smears from tissues collected at surgery or necropsy. The fresh area is subsequently blotted to remove as much blood and tissue fluid as possible (e.g., with a paper towel or a surgical gaze). A clean glass slide is then touched to the brand-new surface. No more smearing is necessary. On the glass slide, the tissue must not be moved, it must only be pressed down and raised immediately. The material is given time to air dry. Avoid rubbing the tissue on the slide since this can lead to cell rupture and nuclear stranding, which will distort the cellular shape. The most frequent issues with touch impressions are non-exfoliative lesions and insufficient specimen blotting. Less cells are gathered, bacterial contamination is more likely, and impression smears only collect cells from the surface of the lesion, which means they may not be typical of underlying pathology.

2. Swabs

Swabs are often only used in situations where other collecting techniques are impractical (e.g., ear canal, vaginal cytology, fistulous tracts). It is advisable to wet the swab with sterile saline if the area to be swabbed is dry. Moreover, a sterile cotton swab should be used to apply a smear on a glass slide if culture is to be performed. They are less beneficial when used to describe impression smearing.

3. Scrapings

In contrast to impression smears of comparable lesions, scrapings are utilised for superficial skin lesions and may be a better indicator of deeper abnormalities. First, gently wipe away any exudates or crusts that are on the surface of the lesion. Then, with a scalpel blade sample are then taken from the lesion's surface, transferred from the blade to a clean glass slide, smeared and let to dry naturally. Similar to imprinting, this technique can also be utilised when imprinting is predicted to produce insufficient numbers of cells for thorough evaluation (e.g., conjunctiva, mesenchymal neoplasia).

5.Tape preparation

A method for evaluation of skin disease is particularly for detecting Malassezia dermatitis and skin mites. A piece of clear, pressure-sensitive tape is placed on the skin lesion. The tape strip is removed and fixed on a microscopic slide containing a drop of blue (counter) stain.

Solid tissue slides preparation techniques

Slide-over-slide technique ("squash") preparation

For the squash preparation, material collected by one of many techniques (e.g., aspiration or non-aspiration fine-needle biopsy, scrapings) is placed in the middle of a clean glass slide (smear slide). A second glass slide (spreader slide) is placed over the sample perpendicular to the smear slide. The sample will begin to spread out due to the weight of the spreader slide. Once the sample has begun to spread, the spreader slide is gently drawn across the sample slide, smearing the sample One must be careful not to put any downward pressure on the spreader slide. Excessive pressure will cause cells to rupture. The slide-over-slide technique works well for spreading samples from nonfragile tissues (e.g., carcinomas). However, squash preparation tends to rupture excessive numbers of cells from more fragile tissues (e.g., lymph nodes). Once spread, the material is allowed to air-dry; No fixative is needed.

Fluid cytology (exfoliative cytology) sample collection techniques

Catheterization

Catheterization means collecting samples by performing various types of washes (e.g., bronchoalveolar lavage, transtracheal wash. Volume permitting, fluid aliquots should be collected into EDTA-containing tubes and sterile/plain tubes. Smears should also be made at the time of sampling. EDTA prevents coagulation and therefore allows for accurate cell counts to be performed when required.

Centesis

Centesis means aspiration of fluid or air from the body cavity by tabbing with aspirator, trocar, or needle. Sample is hold in the same manner as catheterization.

Fluid cytology (exfoliative cytology) slide preparation technique

Blood smear technique

The collected material is placed near one end of a glass slide (smear slide). The spreader slide is tilted to an angle of approximately 45 degrees, pulled backwards into the material and once

the material has dispersed along the width of the spreader slide, the spreader slide is smoothly, steadily and rapidly slid forward. The smear ends with a feathered edge of material. As a general rule, the more material placed on the specimen slide, the slower the spreader slide is slid forward and the more acute the angle between the spreader and specimen slide, the longer the smear will be. The blood smear technique has much less shearing force than squash preparation and causes less cell rupturing. However, blood smearing does not spread cells as well as using slide over slide.

Star fish preparation (needle spread) technique

A material collected by fine needle biopsy is placed in the centre of a glass slide and the needle is used to drag/tease the material outwards in multiple directions to produce a star/starfish shaped smear with multiple projections. Many areas of the smear will be too thick for evaluation, however, there is usually multiple cell monolayer regions present on the smear that should be acceptable for cytological assessment. This technique minimizes the amount of trauma to fragile cells and is useful if only a small volume of material is aspirated. It is used in both solid and liquid cytology slide preparation techniques.

Line smear's technique/role preparation

The line smear technique is useful for concentrating cells in a fluid sample when the sample cannot be centrifuged to make smears from the sediment. A drop of fluid is placed near one end of a glass slide (smear slide). A second slide (spreader slide) is placed on the smear slide at a 45-degree angle and backed up until it contacts the drop of fluid. Moderate downward pressure is applied, causing the nucleated cells to follow just behind the spreader slide (e.g., pulling out to end of smear). The spreader slide is then pushed forward as of making a blood smear, except a feathered edge is not created. After being advanced about two-thirds to three-fourths the distance required to make a smear with a feathered edge, the spreader slide is stopped and then lifted directly up. This creates a line at the end of the smear that has a much higher concentration of nucleated cells.

Cytological stains

Romanowsky-type stains (Wright's, Giemsa and Diff-Quick stain)

Romanowsky stains are inexpensive, easy to use and they are **readily** available to veterinary practitioners. They provide good nuclear **detail**; excellent cytoplasm detail and infectious organisms are readily **visualized**. In clinical practice, the most cost effective, quickest and **easiest** stain to use is the Diff-Quick stain.

Papanicolaou stains

Papanicolaou stains provide excellent **nuclear** detail and adequate cytoplasmic detail, however, they are time **consuming** and impractical for in-clinic usage. Supravital stains provide. excellent nuclear detail but poor cytoplasmic detail and are typically reserved for evaluation of reticulocyte identification (peripheral blood smear) or for evaluating the presence of poorly granulated mast cells.

Microscopic evaluation

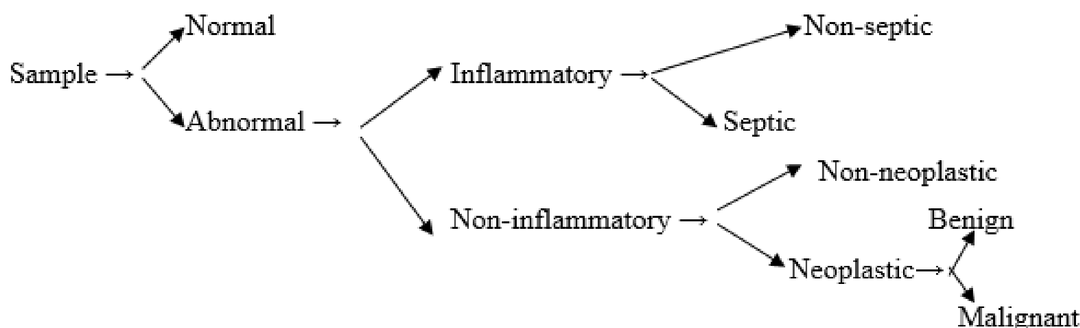
The light microscope is the basic tool that is used to observe objects **too** small to be seen with the unaided eye. Objects or specimens are **magnified** up to about one hundred times and as such could be observed **using** light microscope. The ability to distinguish between two objects **as** separate entities is referred to as resolution. The first objective or **lens** normally used in microscopic work is X10, Medium objective X20, **high** power objective X40 and oil immersion X100.

Applications of Cytology in the Diagnosis of Animal Diseases

Cytology is an inexpensive yet powerful diagnostic tool that allows for rapid diagnosis of many common diseases. There is great potential for application of cytological diagnosis to variety of veterinary and medical conditions. Some of most important application being applied in veterinary medicine has discussed below –

Cytological diagnosis of tumour

A uniform population of cells suggests the mass is benign, whereas variation in cells suggests malignancy. An important exception is lymphoma (lymphosarcoma), which consists of a fairly uniform population of lymphoblast, whereas lymphoid hyperplasia typically has marked variation due to the mixed population of small lymphocytes, lymphoblast, and plasma cells. Nuclear criteria of malignancy are considered diagnostic, whereas cytoplasmic criteria of malignancy are only supportive of malignant neoplasia. It is important to find more than three of the following nuclear criteria of malignancy in a few cells to many cells to call a mass malignant neoplasia. The cytological criteria of malignancy are basically based on nucleus and cytoplasm of cells under neoplastic transformation.



Easily recognized general and nuclear criteria of malignancy

CRITERIA	DESCRIPTION
General Criteria	
Anisocytosis and macrocytosis	Variation in cell size. With some cells > 1.5times larger than normal
Hypercellularity	Increased cell exfoliation due to decreased cell adherence.
Pleomorphism (except in lymphoid tissue)	Variable size and shape in cells or the same type
Nuclear Criteria	
Macrokaryosis	Increased nuclear size. Cells with nuclei larger than 10 u in diameter suggest malignancy
Increased nucleus cytoplasm ratio	Normal non-lymphoid cell usually has a N: C of 1:3 to 1:8 depending on the tissue ratios
Anisokaroysis	Variation in nuclear size. That is especially important if the nuclei of multinucleated cell vary in size.
Multinucleation	Multiple nucleation in a cell. This is especially important if the nuclei vary in size
Increased mitotic figures	Mitosis is rare in normal tissue.
Abnormal mitosis	Improper alignment of chromosomes.
Coarse chromatin pattern	The chromatin pattern is coarser than normal. It may appear ropy or cord – like
Nuclear molding	Deformation of nuclei by other nuclei within the same cell or adjacent cells

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Prognosis of Neoplasms

Cytopathologic interpretations are helpful in prognosis of cancer in animals specially in urinary tract neoplasms, lymphoma, ovarian tumour, mammary gland tumour, horn cancer and tumours of reproductive tract.

Diagnosis of infectious diseases

It is helpful in diagnosis of various infectious diseases of animals, including bacterial and viral diseases e.g., infection in claves. One can demonstrate rotavirus antigen in desquamated cell in diarrhoeic feces.

Cytogenetics

Cytopathologic diagnostic procedures can be employed for chromosomal studies, including karyotyping and aberrations in chromosomes.

Other ancillary studies which could be used on cytological material

Basically, all ancillary studies can be done using cellular material obtained either from exfoliative or FNA technique. These include immunohistochemistry, flow cytometry, molecular pathology studies and electron microscopy *etc.*

Diagnostic Pitfalls

Diagnostic pitfalls can still occur and are usually due to -

Poor collection technique

This can occur when the appropriate slides or containers with appropriate fixatives are not used at the time of the procedure. This can be resolved by consulting with the pathology/ cytopathology department for help.

Poor fixation

This is sometimes seen when there is no experience with cytopathology material preparation and collection. Communication with your pathologist is recommended.

Inflammatory changes

Sometime extensive inflammation may obscure cellular details and prevent appropriate interpretation. To avoid this problem, treating the patient and repeating the procedure afterwards is recommended.

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Cellular changes related to radiation and/or chemotherapy

This issue comes up if the patient had already been diagnosed with malignancy and was treated with chemotherapy and/or radiation therapy. Certain changes are induced by these treatment modalities. To decrease the pitfalls from these changes, appropriate and detailed history should be given by clinicians and awareness of the changes by the pathologist should be taken into consideration.

Atypical cellular changes related to haemorrhage, infarction, or necrosis

Can be problematic. Awareness of these changes by the cytopathologist is very helpful to prevent both false positive and false negative diagnosis. Having a pathologist/cytopathologist at the time of the procedure or performance of the procedure by a pathologist will help alert the pathologist to these changes.

Conclusions

Utilizing the science of cytopathology whether exfoliative or FNA is cost effective, fast, simple and accurate. With the recent improvements in technical aspects and the appearance of cell block technique in cytopathology, the old gold standard of "must have tissue to make an accurate diagnosis" is rapidly changing.

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