INTRODUCTION AND REFERENCES
Cytologic evaluation of specimens collected from diseased ocular structures can be a valuable aid in both the diagnosis and management of ocular disease. Although cytologic analysis may provide a diagnosis of certain diseases (e.g., eosinophilic keratoconjunctivitis in cats), it is often used in conjunction with other tests, e.g., culture and biopsy, to optimize diagnosis and management. Proper collection (including appropriate cautions when sampling damaged tissue), sample processing (including concentration techniques), slide preparation, and staining are prerequisite to obtaining accurate and useful information from the microscopic evaluation. If possible, several slides containing adequate sample volume should be prepared to permit use of different stains and IFA, if indicated. If you are sending the slide(s) to a cytopathologist, it is essential to identify the source of the specimen, e.g., cornea or conjunctiva. Also, if you have stained one of the slides, be sure to include it with the slides you send because the first slide prepared often contains the best material for evaluation. If you are examining the specimen yourself, be familiar with the normal cytologic appearance of the site you have sampled so you can distinguish normal from abnormal cell types and morphologies. Several excellent cytology atlases with chapters on ocular cytology contain both textual descriptions and photomicrographs of normal and abnormal cytology, as well as diagrammatic descriptions of techniques for collection of samples. These atlases include:


STAINS
Romanowsky stains (Wright, Wright-Giemsa, and Giemsa stains) are standard and, in general, are excellent for revealing morphologic characteristics of cells, organisms, and other structures.
The major staining artifact to recognize is stain precipitate that can mimic clusters of cocci. The quick stains (such as Diff-Quik®) are often adequate, but do not stain cytoplasmic features as well as the parent stains. In some instances, for example, mast cell granules do not stain with the quick stains, and the presence of these cells may go undetected if other stains are not used. Also, quick stains must be maintained well or the stains themselves may contain organisms, such as Malassezia, from previously stained specimens or contamination. Other stains that may be used as adjuncts include Gram stain, to determine if bacteria are gram-positive or gram-negative (these can be tricky to read), and stains for fungal organisms, including periodic acid-Schiff (PAS) and Gomori’s methenamine silver stain.

CYTOLOGIC EVALUATION

GENERAL CONCEPTS AND FINDINGS
Ocular specimens are often small in volume, and, therefore, it is easy to examine the entire sample. When identifying cell types, it is important to look in an area where individual cell morphology can be discerned. However, don’t ignore the thick wads of material, often consisting of clustered epithelial cells or necrotic material, found on the slides. These thick areas tend to be understained, and cells with granules that stain more readily than other components (mast cells and eosinophils), naturally pigmented elements (melanin), bacteria, and fungal hyphae may be visualized embedded within or sitting on top of the thick wads.

- Observe the cellular pattern (describe the cells), other structures, and background in an organized way, recognizing artifacts, and then categorize your findings as:
  - Normal: Observer should be familiar with the normal cytologic characteristics of the tissue sampled.
  - Inflammation: Observer should recognize the type(s) of inflammatory cells that are present in order to classify the inflammation and, possibly, to determine the nature of the pathologic process. The combination of cytologic examination and culture is most effective for diagnosing and managing bacterial diseases.

Classification of inflammation:
1) Neutrophilic – synonyms include suppurative, purulent
2) Eosinophilic
3) Lymphocytic/plasmacytic
4) Mixed, including pyogranulomatous
5) Granulomatous

Pathologic processes:
1) Infectious (bacterial, viral, fungal, parasitic)
2) Allergic (hypersensitivity)
3) Antigenic stimulation
4) Immune-mediated  
5) Necrotic

Normal elements of the tissue may also be present.

- **Neoplasia**: Observer should be able to identify both 1) the cell types (epithelial, mesenchymal or connective tissue, and round cells) and 2) the cytologic features of benign and malignant tumors. Examples include squamous cell carcinoma, lymphoma, mast cell tumor, lacrimal gland tumors, and melanoma.

It is important to recognize that neoplasms can induce an inflammatory response.

- **Non-neoplastic mass lesions**: Observer should be familiar with the cytologic characteristics of cysts, acute and chronic hemorrhage, and degenerative diseases.

Once a category is identified, a more specific diagnosis may be possible. At the very least, the category can guide additional testing or therapy.

**SPECIAL CYTOLOGIC FEATURES**

For each of these categories, there are some special cytologic features, detailed below, that may give more definition to the pathologic process manifested by the lesion. It is also essential to recognize common artifacts to avoid misinterpretation of findings.

**Morphologic appearance of neutrophils**: Characteristics of the nucleus and cytoplasm of the neutrophils may offer a clue regarding etiology. Nondegenerate neutrophils may be found in both infectious and noninfectious diseases; if neutrophils are degenerate (contain swollen pale nuclei that lose segmentation), bacterial sepsis, especially from toxin-producing bacteria, is probable.

**Morphologic appearance of epithelial cells**: Epithelial cells, especially squamous cells, may have a dysplastic appearance in sites of intense inflammation. It is essential to differentiate these dysplastic cells from a squamous cell carcinoma, especially as this neoplasm can incite a suppurative inflammatory response. Cornification or keratinization, not detected well in Romanowsky-stained samples because keratin is not visualized with these stains, is abnormal in certain structures, such as the cornea, where the epithelial cells are normally noncornified.

**Significance of bacteria**: If bacteria are found within the cytoplasm of neutrophils, they are considered significant rather than contaminants (unless the sample is fluid and preparation of slides has been delayed with the possibility of in vitro phagocytosis of contaminating bacteria). However, depending on the site and method of collection, any bacteria, including those found extracellularly, may be significant. Certainly bacteria are significant if many organisms of a single morphology are found in a site that should be sterile. Even in sites such as eyelids and conjunctiva where “normal flora” have been identified, the finding of bacteria in the face of inflammation should be considered significant unless there is evidence to the contrary.
**Inclusions:** Inclusions may be found in both epithelial cells and inflammatory cells and may be normal elements, artifacts of treatment, or evidence of the pathologic process or etiology.

**Inclusions found in or on epithelial cells:**
- Normal elements: Melanin granules if from pigmented site; if melanin granules are small and fine, they may be mistaken for mycoplasmal organisms; these fine melanin granules have a greenish tint. Surface bacteria sometimes are found on squamous cells from skin.
- Artifacts of treatment: Drug inclusions if from a site exposed to topical ointment
- Etiologic agents: *Mycoplasma spp.*, *Chlamydophila spp.* (elementary and initial bodies)
- Unknown significance: Neutrophils found within cytoplasm of epithelial cells, especially squamous cells

**Inclusions found in neutrophils:**
- Etiologic agents: Bacteria most frequently (rarely fungal organisms, such as *Histoplasma spp.*)
- Pathologic process: Pyknotic nuclear material in neutrophils. The finding of numerous pyknotic neutrophils signifies accelerated apoptosis of these cells. These are not degenerate neutrophils.

**Inclusions found in macrophages:**
- Erythrocytes: Termed erythrophagia; signifies hemorrhage
- Iron pigments: Usually from breakdown products of RBC degradation; signifies chronic or previous hemorrhage (macrophages are termed “hemosiderophages”)
- Cellular debris: Partially degraded nuclei or cells, such as neutrophils; common to find in an area of long-standing inflammation
- Etiologic agents: Infrequent finding. Certain bacteria, such as *Mycobacterium spp.*, fungal organisms, such as *Histoplasma spp.*, protozoa, such as *Leishmania spp.*, and algae, especially *Prototheca*, can be found in macrophages.

**Extracellular material:** It is essential to examine all the material on the slide, even the background substance. Extracellular material includes:
- Bacteria
- Parasites
- Protozoa (*Toxoplasma, Leishmania*)
- Fungal organisms: *Cryptococcus, Blastomyces*, hyphae of *Aspergillus* and other fungi
- Algal organisms: *Prototheca*
- Free granules: Eosinophil granules (rod-shaped in cats), mast cell granules, and melanin granules. These granules may resemble bacterial cocci or rods, at least in shape. With Romanowsky stains, bacteria are usually dark blue, in contrast to the red-orange color of eosinophil granules and the golden brown or greenish color of melanin granules. Mast cell granules, however, can be easily confused with cocci.
• Cell fragments: Stringy fragmented nuclear chromatin, usually an artifact of slide preparation, can resemble hyphae when surrounded by mucus.
• Crystals, e.g., cholesterol crystals from areas of epithelial degeneration
• Stain precipitate may resemble basophilic clusters of cocci.
• Mucus

CYTOLOGIC EVALUATION OF SPECIFIC OCULAR STRUCTURES:

EYELIDS
Normal elements: Cornified, intermediate, and/or basilar epithelial cells, depending on the depth of the sample; ciliated columnar epithelial cells from the palpebral conjunctiva; and meibomian (sebaceous) glands. Organisms, such as Corynebacterium spp. and Staphylococcus spp., may be found in association with eyelids.

Inflammation: Neutrophilic, eosinophilic, or mixed cell blepharitis (infectious, allergic, or immune-mediated). Organisms may be detected in infectious blepharitis. Mast cells and mononuclear cells may also be noted in allergic blepharitis. Eyelid diseases in horses include cutaneous and systemic fungal infections, papilloma (papovavirus), and habronemiasis.

Neoplasia: Sebaceous (meibomian gland) adenoma (adenocarcinoma rare) and epithelioma, feline apocrine cystadenoma (hidrocystoma), cutaneous melanoma/melanocytoma, lymphoma, cutaneous mast cell tumor, cutaneous histiocytoma, papilloma (reactive and viral), squamous cell carcinoma (especially cats), basal cell tumor, peripheral nerve sheath tumor (cats), other infrequent sarcomas, sarcoid (horses)
NOTE: Cutaneous lid mast cell tumors are usually benign in cats; in dogs, they are classified and behave similarly to mast cell tumors elsewhere in the skin. It is very important to define the location of the mass in these cases (cutaneous vs conjunctival) because conjunctival mast cell tumors in dogs have a much more benign biologic behavior and better prognosis than cutaneous mast cell tumors.

Non-neoplastic mass lesions: Chalazion (lipogranuloma of meibomian gland); hordeolum (abscess of meibomian gland); systemic/reactive histiocytosis of Bernese Mountain dogs; cysts

CONJUNCTIVA
Normal elements: Ciliated columnar cells and goblet cells from the palpebral conjunctiva; noncornified squamous epithelium (seen more frequently than columnar cells in conjunctival scrapings), some with melanin granules, from bulbar conjunctiva; if eye treated with topical ointment, may see smooth round basophilic inclusions in cytoplasm of epithelial cells; if sample obtained near fornix, lymphocytes may be present; bacterial cocci may be noted in samples from dogs without evidence of inflammation.
Note: When obtaining scrapings, first remove debris in order to sample tissue.
Inflammation: Mucus usually accompanies exudative lesions.

- Neutrophilic: Neutrophils may be nondegenerate or degenerate (degenerate neutrophils uncommon in cats); neutrophils may be found in squamous cells.
  1) Bacterial (many organisms may cause conjunctivitis; bacteria may be noted cytologically; culture is usually definitive)
    - If cause is *Chlamydophila* spp., organisms may be detected in epithelial cells (initial bodies are 3-5 µ; elementary bodies are 0.5-1 µ and found in aggregates); IFA may be required for definitive diagnosis; infiltrate may also contain mononuclear cells, plasma cells, and multinucleate giant cells.
    - If cause is *Mycoplasma* spp., organisms may be found on epithelial cells; culture may be required for definitive diagnosis.
  2) Viral
    - Distemper (dogs) and herpesvirus (cats); definitive diagnosis is made by IFA staining; viral inclusions usually not seen cytologically. Multiple viruses are causative in horses, including adenovirus in Arabian foals (may see intranuclear inclusion bodies in epithelial cells).
- Eosinophilic/Mast cell
  1) Hypersensitivity disorder: may also see mast cells and free eosinophil and mast cell granules
  2) Some cats may be positive for herpesvirus.
  3) Eosinophilic conjunctivitis may be a single entity or occur in conjunction with eosinophilic keratitis.
  4) Parasitic, e.g., habronemiasis and onchocerciasis in horses
  5) Feline epitheliotropic mastocytic conjunctivitis (also involves nictitating membrane)
- Lymphocytic/plasmacytic
  1) Follicular conjunctivitis resembles reactive lymphoid hyperplasia.
  2) May also be seen in allergic and chronic infectious conjunctivitis
- Lipogranulomatous conjunctivitis in cats

Neoplasia: Papilloma, squamous cell carcinoma, feline conjunctival surface adenocarcinoma (mucoepidermoid carcinoma), melanoma, lipoma, mast cell tumor (can be a mass or diffuse in conjunctiva of dogs; more benign biologic behavior than cutaneous mast cell tumors), lymphoma, hemangiosarcoma, peripheral nerve sheath tumors (cats), other sarcomas

NOTE: Canine conjunctival melanocytic tumors are usually malignant and often recur due to intraepithelial (pagetoid) spread; this is in contrast to cutaneous lid melanocytic tumors, which are often benign. Thus, definition of the location of the mass is critical.

Non-neoplastic mass lesions: Cystic lesions (dacryops, mucocoele [zygomatic]), depotosteroid granuloma, staphyloma, inclusion cysts, granulation tissue (horses), herniated orbital fat (horses)

**NICTITATING MEMBRANE**

**Normal elements:** Cartilage, conjunctiva, seromucous gland, lymphoid tissue (bulbar surface)
**Inflammation:** This site can be involved in diseases of the conjunctiva, such as feline epitheliotropic mastocytic conjunctivitis; follicular hyperplasia; plasmacytic conjunctivitis in German Shepherds; reactive histiocytosis

**Neoplasia:** Both primary and metastatic tumors reported; lymphoma, squamous cell carcinoma, adenoma/adenocarcinoma of gland of 3rd eyelid, melanoma, carcinoma, apocrine adnexoma, peripheral nerve sheath tumor (cats), conjunctival surface adenocarcinoma (cats)

**NASOLACRIMAL APPARATUS**

**Inflammation:** Dacryocystitis

**Non-neoplastic mass lesions:** Cysts (dacrypeps, parotid transposition mucocoele)

**Neoplasia:** Canine orbital multilobular adenoma (see orbital tumors)

**SCLERA/EPISCLERA**

**Normal elements:** Fibrous tunic and fibrovascular

**Inflammation:** Nodular fasciitis/episcleritis (dome-shaped) usually characterized by mononuclear inflammation: lymphocytes, plasma cells, macrophages, few neutrophils

**Neoplasia:** Lymphoma, mast cell tumor, squamous cell carcinoma, limbal melanocytoma, infiltrating intraocular melanoma/melanocytoma

**Non-neoplastic mass lesions:** Onchocerciasis, reactive/systemic histiocytosis, scleral staphyloma (owing to its dark color, may be mistaken for a pigmented melanocytic mass)

**CORNEA**

**Normal elements:** Collagenous stroma covered by noncornified squamous epithelium

**Inflammation:** Keratitis

Ulcerative lesions: bacterial, fungal (rare in cats – infection with *Cladosporium* reported); hyphae may be embedded in clumps of epithelial cells; special stains (PAS, Gomori’s methenamine silver) may be helpful

Eosinophilic keratitis (granular, nonulcerated, gray-white deposits): eosinophils, including free granules, mast cells, sometimes lymphocytes and plasma cells

In horses, equine herpesvirus-2 infections, parasitic lesions, and stromal abscesses (deeper scrape may be required)

Proliferative inflammatory lesion: Pannus (chronic superficial keratitis with mixed inflammation)

**Neoplasia:** Rare in dogs and cats: melanoma, papilloma (virally induced in young dogs), squamous cell carcinoma, sarcomas (hemangiosarcoma, fibrosarcoma, histiocytoma); in horses, squamous cell carcinoma

**Non-neoplastic mass lesions:** Stromal epithelial inclusion cyst (unique to dogs, caused by trauma?): clear acellular fluid

**AQUEOUS HUMOR**

**Normal elements:** Normally acellular

**Inflammation:** Neutrophilic inflammation seen with most causes of anterior uveitis; organisms may be seen: +/- bacteria, Blastomyces, Prototheca, Leishmania
Neoplasia of the anterior uvea: Lymphoma; less common are carcinomas, sarcomas, transmissible venereal tumor, feline myeloproliferative disease
Non-neoplastic lesions: Hyphema (RBCs, hemosiderophages)

**IRIS/CILIARY BODY**
Normal elements: Melanin (oval or round granules)
Neoplasia: Canine uveal melanosis (Cairn terrier), melanocytoma, malignant melanoma; feline progressive iris hyperpigmentation: with features of anisokaryosis and nucleolar variation, may be diffuse iris melanoma; lymphoma (mass lesion or diffuse), adenoma, adenocarcinoma, primitive neuroectodermal tumor (PNET), anterior uveal Schwannoma of blue-eyed dogs, histiocytic sarcoma, metastatic tumors (primary tumors include, in dogs, respiratory carcinoma, oral and digital malignant melanoma, and hemangiosarcoma and, in cats, respiratory carcinoma and squamous cell carcinoma)

**VITREOUS BODY** (examined if opacity noted)
Normal elements: Normally acellular or may contain few RBCs, few spiculate melanin granules from retinal pigmented epithelial (RPE) cells, and granular background
Inflammation: Endophthalmitis: bacterial (neutrophilic), traumatic (neutrophilic), fungal (neutrophilic) caused by Blastomyces, Cryptococcus, Histoplasma, algal (Prototheca); phacoclastic inflammation; intraocular xanthogranuloma secondary to hyperlipidemia (usually seen in diabetic hyperlipidemia Miniature Schnauzer dogs)
Neoplasia: Cats – post-traumatic sarcoma, chondrosarcoma, lymphoma; dogs – osteosarcoma, lymphoma, histiocytic sarcoma
Other: Hemorrhage (bleeding disorders, retinal detachment, intraocular tumor, hypertension, rickettsial disease)

**ORBITAL MASSES** (typically evaluated by retrobulbar fine-needle aspiration)
Inflammation: Orbital cellulitis and abscess, foreign body, osteomyelitis, extension of sinusitis, oropharyngitis, fungal infections

Neoplasia: Include squamous cell carcinoma (most common), salivary gland carcinoma, lacrimal gland adenoma/adenocarcinoma, canine lobular orbital adenoma, lymphoma, plasmacytoma, melanoma, osteoma and osteosarcoma, multilobular tumor of bone, hemangioma, fibrosarcoma, chondroma and chondrosarcoma, feline restrictive orbital myofibroblastic sarcoma (FROMS), peripheral nerve sheath tumor, rhabdomyosarcoma, optic nerve menigioma, canine orbital hibernoma, and extension of neoplastic disease from sinus or oral cavity

Non-neoplastic lesions: Hematoma; post-enucleation orbital lesions include conjunctival epithelial inclusion cysts, frontal sinus osteomyelitis, mucocoele, and empyema