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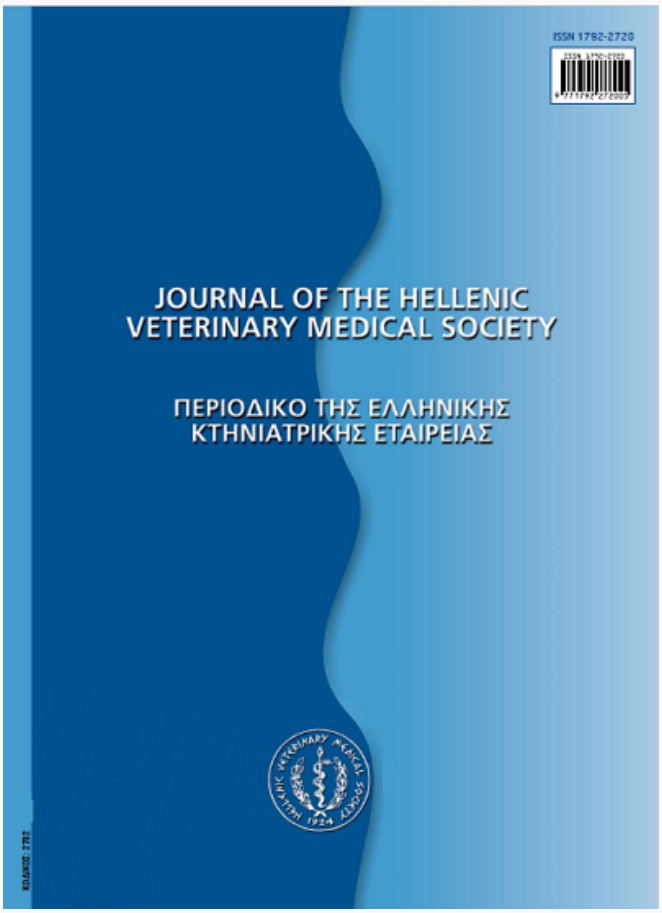


# Η κυτταρολογική εξέταση του επιπεφυκότα στον σκύλο και τη γάτα: δειγματοληψία, διαγνωστικές τεχνικές και ευρήματα

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Review article
Ανασκόπηση

# Conjunctival cytology assessment in dogs and cats. Sampling, diagnostic techniques and findings

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# Η κυτταρολογική εξέταση του επιπεφυκότα στον σκύλο και τη γάτα: δειγματοληψία, διαγνωστικές τεχνικές και ευρήματα

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**ABSTRACT.** The conjunctiva provides a physical and physiological barrier against microorganisms and foreign bodies and also contributes to the ocular immunological defense. It constitutes a straightforward and accessible tissue for sampling and examination. Sampling indications include: changes in color, surface irregularities, thickening, or masses, ocular discharge and the identification of infectious organisms. Samples for conjunctival evaluation may be collected with exfoliative or abrasive techniques, aspiration, impression and conjunctival biopsy. The most commonly used and clinically useful laboratory methods for the assessment of conjunctival specimens are: microscopic examination of cytological preparations, culture and susceptibility testing, live virus isolation, polymerase chain reaction, direct immunofluorescent antigen test and histopathological examination for snip biopsies. Findings like inflammatory or neoplastic cells, cellular alterations, inclusion bodies and microorganisms, offer valuable information not only for localized ocular disorders, but for systemic diseases as well.

***Keywords:*** conjunctiva, sampling techniques, diagnostic methods, cytology, findings, dog, cat

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**ΠΕΡΙΛΗΨΗ.** Ο επιπεφυκότας λειτουργεί ως φυσικός και φυσιολογικός φραγμός έναντι μικροοργανισμών και ξένων σωμάτων, συμμετέχοντας ταυτόχρονα στην ανοσολογική άμυνα του οφθαλμού. Αποτελεί ένα εύκολα προσβάσιμο βιολογικό υλικό για δειγματοληψία και εξέταση. Η συλλογή του κυτταρολογικού υλικού συνιστάται σε παρουσία οφθαλμικού εκκρίματος, ερυθρότητας ή μάζας καθώς και σε πιθανή εντόπιση παθογόνων παραγόντων. Στις τεχνικές συλλογής, ανάλογα με τις εξετάσεις που θα ακολουθήσουν και τις πιθανές επιπλοκές, περιλαμβάνονται κυρίως τα ξέσματα, η παρακέντηση με λεπτή βελόνα, τα εντυπώματα και η λήψη ιστοτεμαχίου. Οι διαγνωστικές μέθοδοι που συνήθως εφαρμόζονται είναι η μικροσκοπική εξέταση του ληφθέντος κυτταρικού υλικού, η καλλιέργεια και το αντιβιόγραμμα, η δοκιμή απομόνωσης ιού, οι μοριακές τεχνικές (PCR), η δοκιμή άμεσου ανοσοφθορισμού για την ανίχνευση αντιγόνου καθώς και η ιστοπαθολογική εξέταση των ιστοτεμαχίων. Τα ευρήματα των εξετάσεων αυτών όπως η παρουσία φλεγμονικών, νεοπλασματικών ή άλλων μη τυπικών κυττάρων, ενδοκυτταρικών έγκλειστων αλλά και η ανίχνευση παθογόνων μικροοργανισμών, συνεισφέρουν στη διάγνωση όχι μόνο παθολογικών καταστάσεων του οφθαλμού αλλά και συστημικών νοσημάτων.

***Λέξεις ευρετηρίασης:*** επιπεφυκότας, τεχνικές δειγματοληψίας, διαγνωστικές μέθοδοι, κυτταρολογία, ευρήματα, σκύλος, γάτα

## INTRODUCTION

The conjunctiva is the thin, semi-transparent mucous membrane covering the eyelids (palpebral conjunctiva), the globe (bulbar conjunctiva) and the entire third eyelid (nictitating conjunctiva). It is variably pigmented and normally appears smooth and moist. Bright, red blood vessels are apparent in non-pigmented areas, indicative of its prolific vascular supply (Maggs, 2008).

The conjunctiva plays a significant role in preventing the desiccation of the cornea and in increasing the mobility of the eyelids and the globe. In addition, it constitutes a straightforward, accessible tissue for sampling and examination, as well as a convenient site for administration of medications (Bauer et al., 1996). Interestingly enough, even though the conjunctiva is the most exposed of all the mucous membranes in the body, it does not stand unprotected. The only lymphatic drainage of the eye is situated in the conjunctiva. On top of that, beneath the upper and lower eyelids lies the conjunctival sac, where mucin is produced. Mucin provides a physical and physiological barrier against microorganisms and foreign bodies (Samuelson et al., 1984) by trapping and disposing both debris and bacteria and by providing a medium for adherence of immunoglobulins (i.e., immunoglobulin A) and microbicidal lysozymes (Nichols et al., 1983). This latter function is essential, considering that conjunctival sacs house considerable microbial flora, including many potential pathogens (Samuelson et al., 1984).

## SAMPLING INDICATIONS

There are several clinical manifestations suggesting that sampling of the conjunctiva should be attempted. These include: changes in color (attributed to hyperemia, anemia, icterus or melanosis), any surface irregularities, thickening, or masses, inadequate or excessive surface moistness, conjunctival edema (chemosis), subconjunctival hemorrhage or emphysema and ocular pain (blepharospasm, rubbing) (Maggs, 2008). In addition, among the primary goals when obtaining cytological samples are the characterization of an ocular discharge (serous, mucoid or purulent), the assessment of inflammatory or neoplastic cells and the identification of infectious organisms involving these surface tissues. Finally, collection and evaluation of conjunctiva cells is encouraged in severe, progressive or recurrent conjunctival lesions and in those cases that are resistant to empirical treatment (Young, 2014).

## SAMPLING TECHNIQUES

Samples for conjunctival evaluation may be collected with exfoliative or abrasive techniques, aspira-

tion and impression cytology. These methods should supply material in adequate amounts for assessment, preserve morphologic integrity and not be uncomfortable or painful to the animal. Additional requirements are operational simplicity and minimum induced-trauma (Bolzan et al., 2005).

The three techniques routinely employed when collecting surface cells are sampling with swabs, spatulas and cytology brushes. A comparison among these reveals their relative advantages and disadvantages, as shown in Table 1 (Bauer et al., 1996; Willis et al., 1997; Maggs, 2008).

**Table 1.** + = poor, +++ = good

| Characteristic | Swab | Spatula | Cytobrush |
|---|---|---|---|
| Total Cellularity | + | +++ | +++ |
| Cellular Integrity | +++ | + | +++ |
| Cellular Distribution | +++ | + | +++ |

Cytological assessment of the conjunctiva is preferably made from freshly derived cells. Therefore, the ocular surface should be rinsed to remove mucus and debris that often conceal the primary lesion. Prior to removing the external debris contained in the conjunctival sac, it is suggested that imprints are made in case this material holds diagnostically essential information (Young, 2014).

**Swabs**

Surface samples are collected by gently rolling a sterile swab across the conjunctival fornix (Fig.1). This is a simple technique and topical anaesthesia is rarely required since it is well tolerated by patients. The number of harvested cells tends to be insufficient for a thorough cytological assessment. However, cellular integrity is well preserved and cells are spread in an even monolayer (Bauer et al., 1996, Willis et al., 1997).

This technique is commonly employed for collection of microbial samples. Pre-moistened swabs, either with proper culture media or sterile saline, are more likely to yield viable organisms. Care should be taken to avoid contact of the swab with the lid margin or facial skin in order to minimize the risk of contamination (Maggs, 2008).

**Spatulas**

Scrapings performed with spatula produce highly cellular samples. However, the cells may clump together, making microscopic examination more difficult (Bauer et al., 1996; Willis et al., 1997). The technique involves conjunctival scraping performed gently with a flat, round-tipped spatula so as not to abrade surface cells that may be diagnostically important (Young, 2014). Conjunctival scrapings are best performed using a Kimura platinum spatula or alternatively, the blunt end of a scalpel blade (the edge closest to the scalpel blade handle) (Bauer et al., 1996; Willis et al., 1997; Bolzan et al., 2005). Swift scraping movements in the same direction until a small drop of fluid accumulates on the edge of the instrument, will harvest enough cellular material for assessment (Fig. 2). Caution is advised, so as not to rupture the globe due to manipulation during the scraping procedure. If the entire conjunctival surface is involved, sampling from the lower eyelid is preferred for convenience reasons.

Collection of conjunctival samples may require administration of topical anaesthetic and when needed, sufficient physical or chemical restraint to avoid any injury to the eye. Due to the highly vascular nature of the conjunctiva, a relatively prolonged application of a topical local anaesthetic may become necessary. This is accomplished by applying a cotton-tipped applicator soaked in proxymetacaine or proparacaine to the conjunctival surface for 20-30 seconds. After 1–2 min, a cotton-wool tip may be applied on the medial canthus to absorb any excess of anaesthetic and the inferior tear lake (Bolzan et al., 2005).

**Cytobrushes**

It has been described that nylon-bristled cytobrushes for collection of conjunctival cytology specimens

from veterinary patients, form more even monolayers and result in superior cell quality and yield, when compared to swab samples (Bauer et al., 1996; Willis et al., 1997; Perazzi et al., 2017). They do tend to be less cellular than those acquired by scraping but this technique is superior in safety and patient tolerance. The brush is carefully rolled over the palpebral conjunctiva after pulling down the lower eyelid (Fig. 3). A topical anaesthetic may be applied prior to sampling to ensure patient compliance.

### Impression

Impression allows the obtainment of conjunctival epithelium components with a good preservation of morphologic features. However, it will not offer clinically significant advantages over scrapings or cytological brush samples, both of which should collect a more satisfactory number of cells from deeper in the epithelium and the superficial stroma. This technique concerns cells that exfoliate with ease, therefore it is better suited when investigating superficial conjunctival disease (Bolzan et al., 2005; Perazzi et al., 2017). Besides using a clean glass slide, conjunctival imprints employing filter strips have been reported in dogs (Young, 2014). The cellulose acetate filter paper in particular, is pressed firmly against the area to be sampled and then peeled away so that exfoliated epithelial cells and surface inflammatory cells are examined (Fig. 4).

### Fine needle aspiration

Fine-needle aspiration is an essential method for assessing conjunctival masses. The technique, identical to the one used for skin masses at other sites, provides an excellent yield from lesions that shed cells relatively freely, especially round cell neoplasms, granulomas and abscesses. The risk of ocular penetration is avoided with adequate physical (or less commonly, chemical) restraint and by ensuring that the needle is always directed away from the globe.

### Biopsy

When standard diagnostics are unrewarding, conjunctival biopsy may be performed on tissues that are too deep to be sampled with the aforementioned cytological methods, or when tissue architecture, rather than individual cellular morphology, is considered to be of value diagnostically. Good samples for histopathological evaluation offer greater amounts and often better preserved cells than does cytology and are more likely to lead to an accurate diagnosis (Young, 2014).

The area of conjunctiva to be sampled is anaesthetized, the eyelid is everted and delicately elevated using a fine-toothed forceps. A small snip biopsy of conjunctiva and subconjunctiva is then resected from its base using small tenotomy scissors (Maggs, 2008). Ocular tissues are very delicate and require smooth handling during the procedure. Normally, hemorrhage is minimal and no sutures are required. Gentle pressure may be applied to the conjunctival wound, that is usually healed without complications. An impression smear of the sample prior to fixation, may offer valuable diagnostic information until results from the histopathological examination become available (Young, 2014).

## DIAGNOSTIC METHODS

Microscopic examination is one of the most important and cost-effective laboratory procedures that are often underutilized during initial diagnostic investigations. Collected samples may be suspended in a sterile solution for testing by polymerase chain reaction (PCR), assessed with direct immunofluorescent staining of a conjunctival scraping or submitted for culture. Scrapings and fluid aspirates can also be applied in a sterile manner to a pre-moistened swab, for microbiological assessment.

### Microscopic examination

Exudative or exfoliative features of conjunctival specimens may supply essential data for a more informed diagnosis. Bacterial, fungal, viral, allergic, degenerative or neoplastic diseases could be determined by cytological evaluation of the conjunctiva (Naib et al., 1967; Young, 2014). Specifically microscopic examination of smears, scrapings, imprints and aspirates may assist in determining any cellular alterations and inclusion bodies and often permits direct observation of organisms, their number and morphology, as well as associated host cellular responses (Maggs, 2008). This information may be

used as a basis for initiating an appropriate treatment plan and assessing the clinical significance of subsequent culture results.

Once collected, samples are gently spread, as thinly as possible, on to a clean microscope slide. The aim is to create a monolayer of cells on the slide, with minimal disruption of cellular morphology. Air-dried slides are then stained appropriately for thorough assessment: modified Wright-Giemsa stains are used for rapid, overall screenings, while Gram stains are often selected for easier detection of smaller organisms, such as bacteria.

**Normal findings**

Cytological examination of specimens from normal conjunctiva reveals sheets of non-keratinized epithelial cells with large, round, homogeneous nuclei and abundant cytoplasm, possibly with melanin granules (depending on coat color) (Lavach et al., 1977; Maggs, 2008). The inner epithelial layer of the eyelid is composed of pseudostratified columnar epithelium and interspersed goblet cells. (Fig. 5 and Fig. 6) The bulbar conjunctiva is composed of stratified squamous epithelium. In most conjunctival samples, nucleated squamous cells are more numerous than columnar cells, and they appear round to cuboidal in shape (Young, 2014). Keratinized epithelial cells are uncommon. Occasionally bacteria may be seen, mainly of the gram-positive type (Lavach et al., 1977). Some white blood cells can also be seen, but basophils and eosinophils are always abnormal. The conjunctival fornix contains lymphoid tissue, however, without clinical signs of conjunctivitis the observation of lymphocytes or plasma cells among epithelial cells, is of little diagnostic importance (Young, 2014). Other routine structures found on conjunctival cytology include mucin strands or plugs.

**Abnormal findings**

Normal, non-keratinized epithelial cells may become keratinized following prolonged exposure associated with ectropion and lagophthalmos. Keratinization may also occur with keratoconjunctivitis sicca (KCS), vitamin A deficiency, and irradiation. An increase in the number of goblet cell occurs with KCS, chronic conjunctivitis, and vitamin A deficiency (Murphy, 1988). Chronicity also causes the epithelium to proliferate, creating folds that give it a "velvety" appearance (Maggs, 2008). An atypical cell population (other than nonkeratinized epithelial cells) with or without mitotic features may suggest neoplastic infiltration. However, chronically, multinucleated giant cells are considered a nonspecific change (Lavach et al., 1977).

Degenerative and non-degenerative neutrophils indicate acute infections (Fig 7), especially of bacterial or viral origin (Maggs, 2008). In chronic disease, neutrophils remain the predominant cell type, with an increased number of mononuclear cells (Lavach et al., 1977; Murphy, 1988). Eosinophils and/or mast cells are also indicative of eosinophilic conjunctivitis/keratoconjunctivitis, particularly when they exceed the number routinely seen in a normal peripheral blood smear. Eosinophils are also detected in parasitic infestations and allergic or immune-mediated conjunctivitis, especially in cats. Plasma cells and/or an abnormal population of lymphocytes, are more typical of reactive hyperplasia, allergic, or chronic conjunctivitis (Maggs, 2008). Plasma cells are characteristic of plasma-cell conjunctivitis. Amorphous, fibrillar hyaline-like material is commonly found in lymphocytic conjunctivitis.

Observed bacteria are often large or small cocci and less frequently rods. The dilemma is determining whether they are of primary importance or simply opportunistic.

Cytology of an ocular discharge can assist in distinguishing simple mucous from purulent material, which contains numerous bacteria and neutrophils. A serous ocular discharge in particular is due to an increase in tear production and often related to superficial irritation of the conjunctiva or cornea. Stimulation to the goblet cells may result in exudates containing mucous, which characteristically causes cells to be aligned in rows on the smear. A purulent discharge often indicates a bacterial infection (Maggs, 2008). More specifically, the neutrophilic exudate of canine conjunctivitis often contains bacteria, regardless of the primary cause. On the other hand, the exudate of feline neutrophilic conjunctivitis rarely contains bacteria. When it does, it should be considered a clinically significant finding (Young, 2014).

Fungi are commonly recovered from the eyelids and conjunctiva of normal animals, and they are believed not to be permanent floral residents of the ocular surface but evidence of random environmental exposure. Fungal hyphae stain as linear septate structures with parallel walls, branching at various angles. The presence of fruiting bodies (conidiophores) could allow speciation, while fungal detection and identification can also be achieved by fungal culture or genetic sequencing (Sparagano and Foggett, 2009). Fungal conjunctivitis is very rare in the dogs.

Even though viruses, *Mycoplasma spp*. and *Chlamydiophila spp*. are too small to be detected by means of traditional light microscopy, occasionally, distinctive inclusion bodies may be discerned, especially in acute infections (Maggs, 2008).

Canine distemper inclusion bodies (Fig. 9) may be found in the conjunctival epithelial cells after approximately six days of infection and are seen more frequently in cells originating from the nictitating membrane. However, these inclusions are scarce and are rarely discovered. Therefore, a search for them is of limited diagnostic value (Young and Taylor 2006; Young, 2014).

Feline Herpesvirus (FHV-1) infection is a common cause of feline neutrophilic conjunctivitis. Multinucleate epithelial cells may be found, but intranuclear inclusion bodies are seen rarely, if ever, cytologically (Young, 2014).

*Mycoplasma spp.* may be seen as clusters of small indistinct basophilic 'dots' on routinely stained smears, over the flattened surface of squamous epithelial cells or between cells (Young, 2014). There have been studies claiming cytological examination is less reliable in the diagnosis of mycoplasmosis (Hillstrom et al., 2012).

*Chlamydiophila felis*, an obligate intracellular organism, causes mainly conjunctivitis. The diagnosis may be confirmed by identifying intracytoplasmic inclusion bodies during the acute phase of the disease. These basophilic to slightly purple elementary intracytoplasmic bodies are found in the cytoplasm of squamous epithelial cells while they may also appear as aggregates of coccoid basophilic bodies (elementary bodies) (Hillstrom et al., 2012). In chronic conjunctivitis, intracytoplasmic organisms are present only infrequently (Hoover et al., 1978; Nasisse et al., 1993).

It should be mentioned, that inclusion bodies are not frequently discerned, and failure to detect them does not prove that these organisms are not present. Furthermore, caution is advised when differentiating such inclusions bodies from intracytoplasmic melanin granules (Maggs, 2008) (Fig. 8) while also taking into consideration that in animals treated with topical ophthalmic ointments (particularly neomycin), epithelial cells may possibly contain dense basophilic homogeneous cytoplasmic inclusions (Streeten and Streeten, 1985).

The tumor types associated with the conjunctiva are similar to those that involve the eyelids, and include the following: papilloma, sebaceous adenoma, apocrine (basal cell) adenoma or trichoblastoma, squamous cell carcinoma, histiocytoma, lymphoma, mast cell tumor, melanoma, lipoma and others (Fife et al., 2011).

**Culture and susceptibility (sensitivity) testing**

Microbial flora in the conjunctival sac can be divided into resident and opportunistic pathogenic organisms. Resident bacterial populations are usually isolated from bacteriologic samples of the canine conjunctiva in large numbers. They consist of non-invasive organisms that play an important homeostatic role by competing with pathogenic species for space and nutrients and also by secretion of active substances that limit their ability to colonize the ocular surface. It follows that indiscriminate use or long-term application of antimicrobials and/or corticosteroids may disrupt this balance and predispose to over-growth of pathogens (Gerding and Kakoma, 1990; Maggs, 2008; Wang et al., 2008).

Bacteriological samples should preferably be collected prior to the start of antibiotic administration; however, organisms that persevere in spite of the antimicrobial treatment are also relevant. Similarly, sampling for bacterial culture should precede the application of topical anaesthetics, due to the inhibitory preservatives they contain. On the other hand, it has been reported that it is unlikely these anaesthetic preparations may alter cultures in a clinically relevant way (Champagne and Pickett, 1995).

Results of cytological examination and bacterial culture have been compared, and found to be complementary (Massa et al., 1999). In all cases, better results are expected when sufficient material is available for assessment. Refrigeration, not freezing, of the sample will maintain the number of viable organisms when a delay in testing is anticipated. Bacteria, chlamydiae, mycoplasmas, fungi and viruses have different culture requirements. Swab type, transport medium and storage and transport conditions are factors that should be taken into consideration. For instance, *Chlamydiophila* and *Mycoplasma* require specific transport medium, as these are obligate intracellular organisms. This involves close communication between the examiner and the associated laboratory to which the sample will be sent. On top of that, the clinician should make certain that the laboratory is equipped to test antibiotics that are applied topically, since these are not routinely included in all test panels.

Cultures of normal flora tend to be represented by more than one isolate, and usually appear in light growth, often only in enrichment media. Nevertheless, culture results must be carefully interpreted because differentiation of pathogens and normal flora may often prove difficult.

Bacteria can be cultured from the conjunctival sac of about 40%– 90% of normal dogs. Gram-positive aerobes are the most commonly cultured, with *Staphylococcus spp., Bacillus spp., Corynebacterium spp.,* and *Streptococcus spp*. predominating. Predominant gram-negative isolates recovered from the conjunctival sac in 7%–8% of normal dogs are *Acinetobacter sp., Neisseria sp., Moraxella sp., Pseudomonas sp.,* and *Escherichia coli* (Gerding and Kakoma, 1990; Whitley 2000; Thangamuthu and Rathore, 2002; Prado et al., 2005; Wang et al., 2008).

Bacterial cultures from normal cats' eyes tend to yield organisms approximately half as frequently as those from dogs' eyes.Bacteria cultured from the conjunctival sac of 4%–67% normal cats are principally gram positive: *Staphylococcus sp., Corynebacterium sp., Streptococcus sp.,* and *Bacillus sp*. Predominant gram-negative isolates are *Pseudomonas sp., Chlamydiophila felis, Mycoplasma sp.,* and *Parachlamydia acanthamoebae* (Espinola and Lilenbaum, 1996; Di Francesco et al., 2004; Richter et al., 2010).

Anaerobes are rarely isolated, and susceptibility testing of anaerobic isolates is not commonly performed and may only be required with aspirates and deeper biopsies, particularly from orbital masses.

**Fungal culture**

Despite the fact that the normal ocular surface is home to a wide range of both commensal and transient fungal populations, detection of these organisms in a diseased eye may prompt the clinician to consider treatment with an appropriate antifungal agent. Fungal involvement is otherwise indicated when an appropriate antibacterial treatment has failed to produce the anticipated results, or when the bacterial flora has been altered, following a systemic or local immunosuppression or prolonged use of antimicrobial drugs. Material harvested by conjunctival aspirates, deep biopsies, swabs or scrapings can be submitted for fungal culture at specialist laboratories, but tends to be expensive.

The ubiquitous free-living saprophytic fungi that are most commonly found on the conjunctival surface of normal dogs and cats are *Penicillium sp., Cladosporium sp, Aspergillus sp, Alternaria sp, Fusarium sp.* and related species (Whitley, 2002; Prado et al., 2005; Wang et al., 2008).

**Live virus isolation (VI)**

This method confirms the presence of live virus in a collected sample. It is considered unsuitable for in-clinic use, as it is technically demanding and labor-intensive. The virus replicates on specific cell lines resulting in characteristic cytopathic effect on the cells. The most frequent ophthalmic application for viral culture or virus isolation has been the diagnosis of FHV-I (Young, 2014).VI is a sensitive and specific technique, as long as the viruses are not labile and the sample transport and cultural conditions are optimal. Swabs are collected from the conjunctival surface and then transported in viral and chlamydial transport medium (VCTM). Regarding herpesviruses particularly, it is essential to refrain from calcium alginate swabs and stains such as fluorescein and rose Bengal, due to their inhibitory

effect. Instead, use of Dacron or cotton-tipped swabs is advised. Furthermore, it should be noted that there is a possibility that the use of topical anesthetics prior to sampling may reduce sensitivity (Storey et al., 2002). The diagnosis of FHV-1 infection can be confirmed by virus isolation, however identification of FHV-1 DNA using PCR is the most sensitive and specific technique even though it poses some difficulty because of the relatively high frequency of normal cats reported to test positive for FHV-1 (Stiles et al., 1997).

**Polymerase Chain Reaction (PCR)**

PCR does not require the presence of viable organisms since it detects even minute quantities of DNA. However, this is similarly considered an unsuitable method for in-clinic use because it is technically demanding and it requires great care at all stages of collection, transport and testing, to guard against contamination. It should be noted that the quality of the produced results is relative to the quality of the laboratory.

Conjunctival samples are principally submitted for PCR testing to diagnose *Chlamydiophila felis*, *Mycoplasma spp*. and FHV-1. These are obligate intracellular pathogens, therefore highly cellular samples are more likely to yield positive results. Plain swabs may be used and samples can be suspended in sterile phosphate buffered saline when forwarded for testing.

The use of non invasive sampling, such as collection of conjunctival swabs as a diagnostic tool for the detection of *Leishmania* sp. DNA through PCR has recently been studied and the results showed that the technique is a sensitive and practical method and represents a good option for an early and simple diagno-

**Fig 1.** Sample collection by sterile swab

**Fig 2.** Conjunctival scraping

**Fig 3.** Collection of conjunctival cells by cytobrush

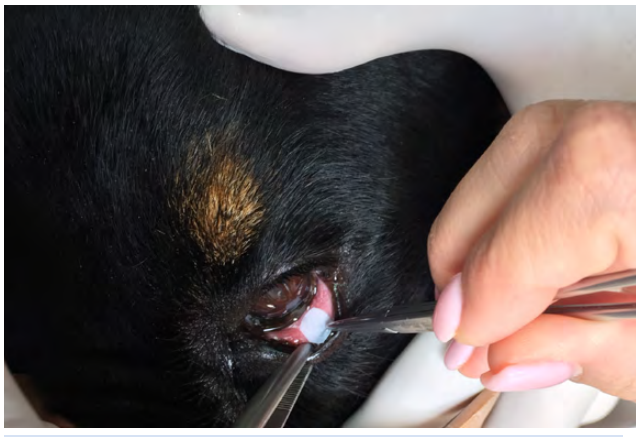

**Fig 4.** Use of cellulose acetate filter paper for sampling

sis of canine *Leishmania* infection in asymptomatic animals, for regular screenings of dogs and for monitoring relapses in drug-treated dogs (Lombardo et al., 2012; Geisweid et al., 2013).

The conjunctiva tends to contain relatively large numbers of bacteria, as well as fungal organisms, often part of the commensal flora of the ocular surface. Since bacteria can usually be readily cultured and standard PCR cannot distinguish transient flora from the one involved in pathogenesis of disease, PCR has infrequent application in their detection.

### Direct Immunofluorescent Antigen Test

Direct Immunofluorescent Antigen testing is a diagnostic aid that can be performed on conjunctival tissue to confirm a viral or chlamydial infection (Maggs, 2008). The technique involves addition of a fluorescently labeled antibody to an air-dried cytological preparation (Fig. 5). The most common agents diagnosed with Direct Immunofluorescent Antigen Test are FHV-1, canine distemper virus (Athanasiou et al, 2018), adenovirus and *Chlamydiophila felis*. False negatives occur when an adequate sample is not obtained. Furthermore, because most of these tests use fluorescein-conjugated antibody to detect FHV-1 antigen within the submitted tissue, topical fluorescein should be avoided prior to collection.

## CONCLUSION

In conclusion, sampling of the conjunctiva should be considered as an essential, non-invasive procedure that produces specimens allowing multiple diagnostic approaches and offering valuable information not only for localized ocular disorders, but for systemic diseases as well.

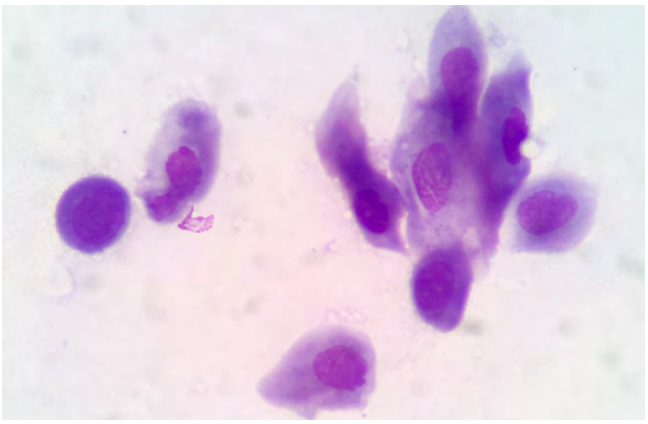

**Fig 5.** Pseudostratified columnar epithelial cells

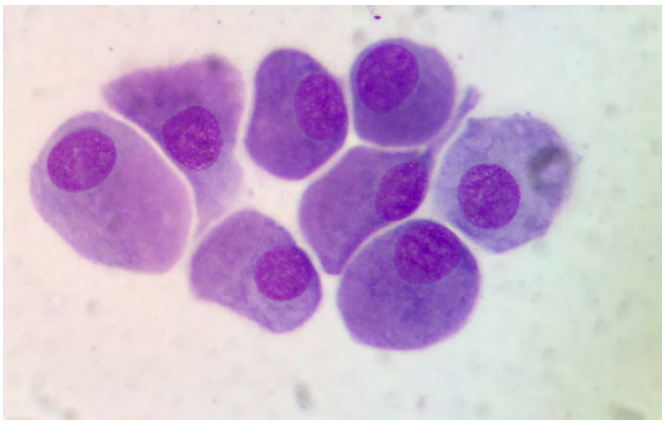

**Fig 6.** Conjunctiva goblet cells

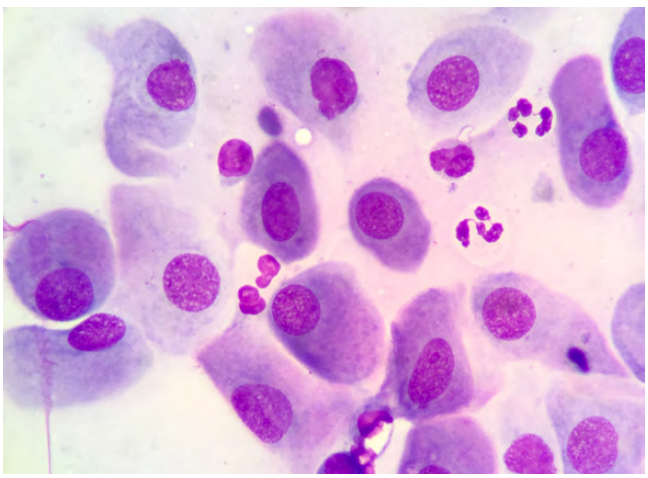

**Fig 7.** Presence of neutrophils in conjunctival cytological preparation

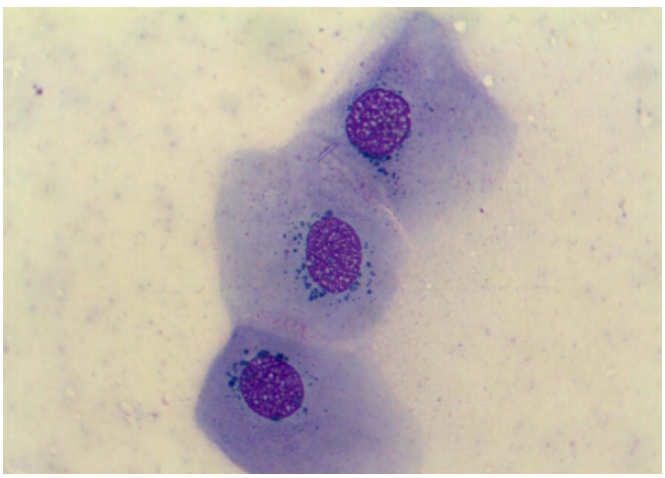

**Fig 8.** Melanin granules as seen in conjunctival epithelial cells

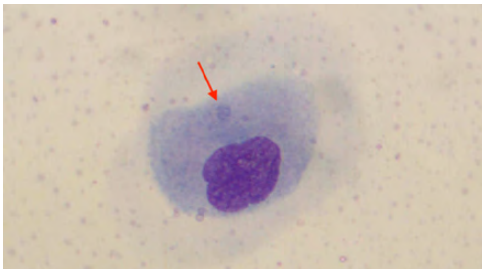

**Fig 9.** Goblet cell with an inclusion body in a conjunctival sample of a dog suspected of distemper virus infection

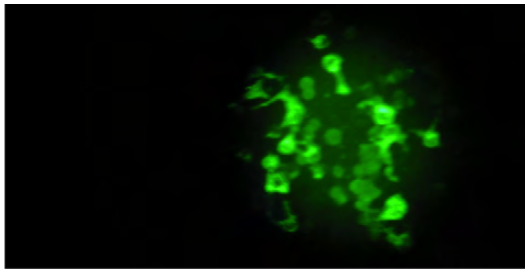

**Fig 10.** Antigen fluorescence of a distemper positive conjunctival sample due to labeled antibody to an air-dried cytological preparation

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