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DRY EYE. IT IS NOT JUST SCHIRMER TEAR TEST

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All components of the ocular surface microenvironment (OSM) play an important role in maintaining the ocular surface (OS) homeostasis. In case of OS disorders, the basic and advanced diagnostic tests are targeted to define the level of involvement of each single lacrimal functional unit (LFU) structure, if any, in the disease process.
Different tests are available to estimate eyelids and meibomian glands, tear film (TF) production, turnover, volume and stability and the OS epithelia. All tests are an adjunct to a complete ophthalmic examination to be performed with all instruments needed. On the light of the data reported in literature and of the personal experience, some tests used for humans have little or no feasibility for animals.

EXAMS

1. EYELIDS AND MEIBOMIAN GLANDS (MGs)

Blinking/lid closure analysis
Blinking is vital in maintaining a healthy OS by cleaning and protecting it. An adequate TF must be continuously reformed by eyelid movements. Hence, it's mandatory to check for the number of complete and incomplete blinks per minute. Video recording may be useful to double check blinking.

Complete blinks
Man: 15/min
Dog: 3-5/min
Cat: 1-5/5min

Excited dogs → 10-20/min complete & incomplete

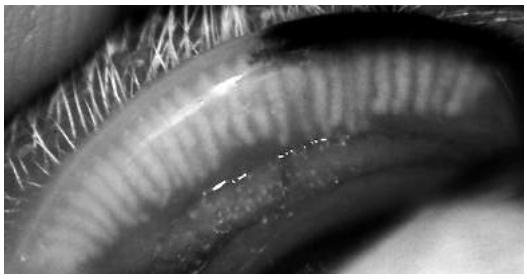
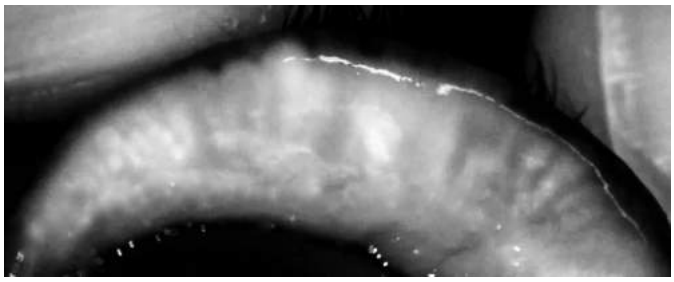
MGs expression
MGs expressibility allows to evaluate meibum quantity, quality and ductal occlusions. Expression is performed as an indicator of MGs function.
In the normal patient, a clear to light yellow oil (meibum) is excreted from the glands when digital pressure is placed on the eyelids. Changes in meibomian gland expressibility may be a valuable indicator of disease.

Meibometry
Quantification of meibomian lipid secretion by meibometry is no longer considered clinically relevant in veterinary medicine since a large range of values has been reported in dogs and because of the test low repeatability.

Meiboscopy
Contact meiboscopy is a transillumination technique applied to the cutaneous side, the eyelid being everted over a light source like a Finoff transilluminator. MGs are observed from the conjunctival surface of the eyelid.

Meibography
Non-contact infrared meibography (NCIM) is a technique to examine MGs by infrared light and document clinical findings by images or videos. OSA-VET® (SBM Sistemi, Torino, Italy) is the ocular surface multipurpose analyzer used for this procedure and for TF interferometry as well.
The main purpose to perform meibography is to detect

Normal *MGD*

clinical signs of meibomian gland dysfunction (MGD) characterized by:

- ductal openings capped by a dome of oil with a tough surface, plugged with inspissated secretion, occluded, displaced posteriorly by a cicatricial process
- gland dilatation, distortion, shortening, atrophy and dropout
- extended, cigar-shaped structures that seem to occupy the position of one or more meibomian glands

Post-production processing allows to calculate the meiboscore to evaluate in detail MGs involvement in pathological processes.

Further tests not commonly used on animals

- **Eyelid sensitivity test**. A Cochet-Bonnet esthesiometer may be applied to evaluate lid sensitivity. In humans an increase of lower eyelid margins sensitivity may be related to elevated tear film osmolarity. No data are available for animals.
- **Lid wiper epitheliopathy test (LWE).** «Lid Wiper» is that portion of the marginal conjunctiva of the upper eyelid that wipes the OS during blinking. The increased friction through blinks in dry eyes and hydrodynamic forces induced by TF viscosity are the main cause of marginal conjunctival distress with consequent staining by fluorescein or lissamine green.
- **Confocal laser scanning microscopy (CLSM)** can be used for the in vivo examination of the eyelid margin and to assess MGs morphological changes.

2. TEAR FILM STABILITY

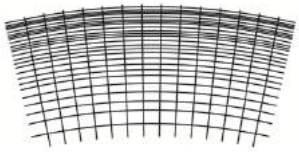
<u>Break-up time (BUT)</u>

To assess TF stability one drop of fluorescein stain is applied to the cornea, the eyelids are closed and then opened and held apart to examine the OS by a slit lamp with blue light. BUT is the time between lid opening and initial TF breaks evidenced as dark lines/spots in contrast to the green background stain. When we evaluate this test we must consider the consistent interference of the drop of fluid from the fluorescein strip (about 17µL, more than the total TF volume).

Dog: normal 14,5 +- 4,1 sec
Cat: normal 12,4 sec (9,1-17,7 sec)

BUT < 10sec = TF instability // BUT < 5sec = dry eye disease (DED)

<u>Non-invasive break-up time (NIBUT)</u>

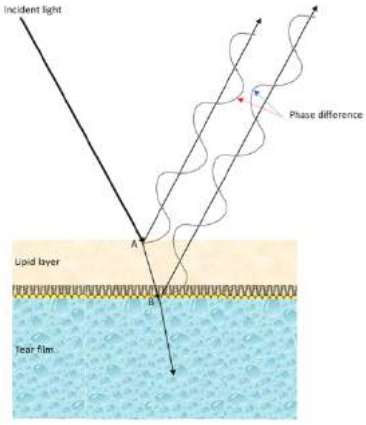


In OS examination non-invasive methods should be always preferred.
An OS analyzer to examine the LL by interferometry is needed. OSA-VET® (SBM Sistemi, Torino, Italy) is the instrument extensively used for this purpose.
NIBUT is evaluated by examining circles and lines of a dedicated grid projected over the cornea forming interferometric patterns. Time from blinking and initial grid

distortion is not influenced by administration of drops and is exclusively a variable of TF composition and OS wettability.
In most cases in animals continuous eye and third eyelid movements prevent NIBUT evaluation although the palpebral fissure is held wide open.

TF interferometry
The TF LL can be visually examined by observing interference patterns generated by light reflected from its front surface (air-lipid boundary) and the lower surface (lipid-aqueous boundary).
LL thickness is evaluated by looking at texture, colour and dynamics of LL patterns and comparing them to conventional grading scales.
A simplified three main patterns grading scale may be used in clinical examination:

- *Light striking a thin film is partially reflected and partially refracted at the top surface (A)*
- *The refracted ray is partially reflected at the bottom surface (B)*
- *The coloured interference fringes are caused by the specular reflection at A and B and the phase difference between the emerging rays*
- *Colour intensity and distribution vary according to the thickness of the film and the indices of refraction of the various media*



- faintly visible homogeneous meshwork pattern (≃15-30 nm)
- compact meshwork pattern, grey waves (≃30-60 nm)
- meshwork with waves and interference fringes (≃60-150 nm)

TF osmolarity variability
TF osmolarity is reported as the single best metric to diagnose and classify dry eye disease in humans. Current measurement techniques are highly variable and not comparable. The most used instruments by veterinary ophthalmologists are TearLab® and i-penVet®. TearLab® collects a 50 nL tear sample and analyzes its electrical impedance while i-penVet® measures the impedance of the saline concentration of the extracellular fluid on the conjunctival surface.

Dog = 318 (296 – 339 mOsms/L)
(337,4 +-16,2 - ARVO)
Cat = 322 (297 – 364 mOsms/L)

In normal conditions, small osmolarity differences over the OS and in the tear meniscus are commonly observed.
In dry eye, particularly in EDE cases, TF evaporation leads to a hyperosmotic shift and osmolarity data over the OS and in the tear meniscus may be consistently different.

Further tests not commonly used on animals

- **TF evaporation rate (TFER).** The TFER has been measured using a number of different techniques and instruments available for humans and in research settings.
- **Thermography.** TF evaporation results in a cooling of the OS. Thermography is evaluated by measuring the absolute temperature and its spatial and temporal changes during the inter-blink period.
- **Fluo-Clearance Test (FCT).** The FCT is performed to detect tears turnover by evaluating tears secretion by STT every 10 minutes and clearance by detecting fluorescein in strips examined by fluorophotometry.

3. TEAR FILM VOLUME

Schirmer Tear Test (STT)

STT is performed by folding the standard sterile Schirmer paper strip at the notch and hooking the folded end over the temporal one-third of the lower lid margin. After 1 minute the wet portion of the strip measures the score.
STT-1, without topical anesthesia, provides an estimation of basal and stimulated reflex tear flow.
STT-2, with topical anesthesia, provides an estimate of basal tear flow. One drop of topical anesthetic is administered first, the excess is blotted away with a swab. STT strip is applied after a few minutes.
Different STT reference scores have been set by several authors. The data reported below are from the chapter "Ophthalmic examination and diagnostics" by Featherstone in the Gelatt Veterinary Ophthalmology textbook.

STT: Dog = 18,64 ± 4,47 to 23,90 ± 5,12 mm/min
Less than 10 mm/min suspicious of KCS if symptomatic
Less than 5 mm/min → KCS

STT: Cat = 14,3 ± 4,7 to 16,92 ± 5,73 mm/min
Wide range of normal values

Phenol Red Thread Test (PRTT)

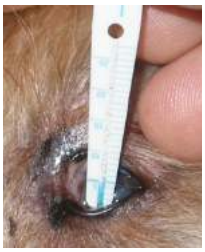
PRTT is performed by placing in the lower conjunctival fornix the 3 mm folded extremity of a 75 mm cotton thread. After 15 seconds the PRTT score is evaluated by measuring the length of thread with colour change.

PRTT: Dog = 34,15 ± 4,45 mm/15 sec
Cat = 23,04 ±2,23 mm/15 sec

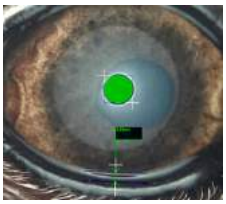
Meniscometry

Strip meniscometry (SM) is a practical method to test tear volume by dipping a polyethylene strip with a 0.4 mm central ditch with blue dye reservoir for 5 seconds into the tear meniscus.
Tear Meniscus Height (TMH) may be also evaluated on selected interferometric images and software processing to make interesting deductions about relationships of tear volume with stability.
In both methods meniscometry should be evaluated 3-4 seconds after blinking.
SM scores should correspond to about half of STT values.

SM: Dog = 9,66 ± 2,15 mm/5 sec
Cat = 10,50 ± 1,20 mm/5 sec



TMH (with normal STT)
DOG = 0,53 ± 0,11 mm



4. TEAR FILM COMPOSITION

TF interferometry
As for TF stability at point 2.

TF osmolarity
As for TF stability at point 2.

Tear ferning test (TFT)
Ferning occurs when the tear film is dried on a slide. The pattern of the tear fern depends on the composition of the tear sample and may be influenced by ambient humidity, temperature, dirt and mucus. The ferning patterns are observed under a polarized light microscope and classified according to a grading scale.

5. OCULAR SURFACE
OS testing is targeted to diagnose and interpret epithelial and surface defects affecting TF stability.

Sodium fluorescein staining
The most common methods to perform OS staining is by moistening a fluorescein sterile strip with one drop of saline and apply the drop to the eye under the upper eyelid without touching the cornea.
In alternative a sterile strip may be placed in an empty syringe prior to drawing up 3 ml of saline.
To avoid false staining interpretations the eye is then rinsed with additional saline.
A positive staining may be:

- **intense** when the exposed stroma is stained in corneal ulcers
- **faint** when hydrophilic substance in intercellular spaces is stained in case of disruption in superficial cell tight junctions or defective cellular glycocalyx
- **weak** due to background fluorescence of healthy corneal epithelial cells

Rose Bengal staining
Sterile strips are used and the same procedure is applied as for fluorescein staining.
Rose Bengal is toxic to healthy corneal epithelial cells in a dose dependent manner and may irritate and damage the OS.
A positive staining occurs when OS epithelial cells are unprotected by secreted mucins or in the presence of altered membrane associated mucins in the glycocalyx, irrespective of the state of cell health.

Lissamine green staining
Sterile strips are used and the same procedure is applied as for fluorescein and rose Bengal staining.
Lissamine green must be left in contact with the OS for a longer time: it's necessary to evaluate staining within 2 minutes with a low intensity light (red filter).
Positive staining of epithelial cells occurs only **if the cell membrane is damaged** irrespective of the presence of mucin.

OS topography by Placido disc

Corneal surface may be better evaluated by applying a Placido disc to the OSA-VET® interferometer. The arrangement of the concentric circles projected over the lipid layer highlights changes in curvature and any defect of the OS, including abnormal TF distribution.

Impression cytology
Corneo-conjunctival cytology may be used to characterize the disease process involving the OS, to identify organisms (bacteria, fungal hyphae, yeast bodies) and epithelial, inflammatory or neoplastic cells.

OS sensitivity

Corneal esthesiometry may be performed by the Cochet-Bonnet esthesiometer.

Further tests not commonly used on animals

- **Lid Parallel Conjunctival Folds (LIPCOF).** The presence of conjunctival folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin, may be related to completeness and speed of the blink and tear film viscosity. LIPCOF is associated with decreased mucin secretion, correlated to LWE.
- **Confocal laser scanning microscopy (CLSM).** CLSM can be used to evaluate in vivo OS damage at a cellular level.
- **OS inflammation tests.** The most useful indicators of OS inflammation that can be tested are:
  - *Conjunctival redness.* It's a consistent sign of conjunctival vascular dilatation and reactive change to pathological stimuli. A grading scale may be used to grade the clinical condition.
  - *Matrix metalloproteinases (MMPs).* MMPs are one of many classes of proteases secreted into the tears in OS disorders. Their level reflects the loss of OS barrier function, since MMPs can destroy tight junctions in the OS epithelium.
  - *Cytokines and chemokines.* Their levels in the tear film reflect the level of epithelial disease.
  - *OS immune markers*. Several ocular surface immune markers indicate a loss of the normally immune-suppressed environment of the OS.
  - *Confocal laser scanning microscopy (CLSM)* has been used to examine cellular changes of the OS morpho-functional unit which correlate to TF inflammatory mediators.

Suggested readings

- Blocker T, van der Woerdt A. A comparison of corneal sensitivity between brachycephalic and Domestic Short-haired cats. Veterinary Ophthalmology 2001, 4, 2, 127-130
- Featherstone HJ, Heinrich CL. Ophthalmic examination and diagnostics in Veterinary Ophthalmology. 2013. Edited by KN Gelatt, BC Gilger, TJ Kern, fifth edition, vol. 1,Wiley-Blackwell, Ames, Iowa, USA
- Hartley C, Williams DL, Adams VJ: Effect of age, gender, weight, and time of day on tear production in normal dogs. Veterinary Ophthalmology, 2006, 9(1):53-57
- Knop E et al. The International workshop of Meibomian gland dysfunction: report of the subcommittee on anatomy, physiology and pathophysiology of the Meibomian gland. IOVS 52,4,1938-1978, 2011
- Leiva M, Peña T. Diseases of the lacrimal system in Slatter's Fundamentals of Veterinary Ophthalmology. 2018, 6th edition. Editors DJ Maggs, PE Miller, R Ofri. Elsevier St Louis
- Miller PE. Lacrimal System in Slatter's Fundamentals of Veterinary Ophthalmology. 2013, 5th edition. Editors DJ Maggs, PE Miller, R Ofri. Elsevier St Louis
- Oria' AP et al. Tear ferning test in healthy dogs. Veterinary Ophthalmology (2018) 21, 4, 391–398
- Pflugfelder SC et al. Management and therapy of dry eye disease: Report of the Management and Therapy Subcommittee of the International Dry Eye WorkShop. Ocular Surface, 5(2):163-178, 2007
- Rajaei SM et al: Strip meniscometry in dogs, cats, and rabbits. Vet. Ophthalmol. 2018 21(2):210-213
- Willcox MD, Argueso P, Georgiev GA et al. TFOS DEWS II Tear Film Report. Ocular Surface, 15, 366-403, 2017

ABBREVIATIONS

ADDE: Aqueous Deficient Dry Eye
BUT: Break-Up Time
EDE: Evaporative Dry Eye
FCT: Fluo-Clearance Test
IVCLM: In Vivo Confocal Laser Microscopy
LIPCOF: Lid Parallel Conjunctival Folds
LL: Lipid Layer
LWE: Lid Wiper Epitheliopathy

MGs: Meibomian Glands
MGD: Meibomian Gland Dysfunction
MMPs: Matrix Metalloproteinases
NCIM: Non-Contact Infrared Meibography
NIBUT: Non-Invasive Break-Up Time
OS: Ocular Surface
PRTT: Phenol Red Thread Test
SM: Strip Meniscometry
STT: Schirmer Tear Test
TF: Tear Film
TFT: Tear Ferning Test
TMH: Tear Meniscus Height