

 ADER Enterprises Inc. aderent.com	1 of 9	SOP #:	ESA - 001
		Revision #:	A
		Implementation Date:	12/Dec/07
Page #:		Last Reviewed/Update Date:	30/Apr /08
SOP Owner:	ADER Enterprises Inc.	Approval:	Emilio Robledo PhD

Procedure for the evaluation of the anterior segment of the eye in dogs and cats

1. Purpose

The purpose of this document is to describe the sequence to be utilized in evaluating the anterior segment of the eye in small species, and whose collected data will serve as a basis for documenting clinical cases and/or clinical studies.

2. Scope

In this document, techniques will be demonstrated so as to successfully perform the following evaluations in the anterior segment in dogs and cats eyes:

- 2.1 Evaluation of visual acuity
- 2.2 Evaluation of lacrimal film utilizing the test named below:
 - 2.2.1 Schirmer Test
- 2.3 Corneal evaluation utilizing the following tests:
 - 2.3.1 Coloration test with Fluoresceín
 - 2.3.2 Coloration test with Bengale Rose
 - 2.3.3 Coloration test with Lisamine Green
- 2.4 Evaluation of the transparency of the lens using the following technique:
 - 2.4.1 Distant direct ophthalmoscopy

3. Prerequisites

To successfully carry out the tests referenced in Section 2 (Scope), one must use the following materials and equipment:

- 3.1 Visual acuity test:
 - 3.1.1 Evaluation room with adjustable lighting.
 - 3.1.2 Low intensity laser light
 - 3.1.3 Cotton balls
- 3.2 Schirmer test:
 - 3.2.1 Sterile graduated strips for Schirmer testing, **SON-STRIPS** brand or equivalent.

 ADER Enterprises Inc. aderent.com	2 of 9	SOP #:	ESA - 001
		Revision #:	A
		Implementation Date:	12/Dec/07
Page #:		Last Reviewed/Update Date:	30/Apr /08
SOP Owner:	ADER Enterprises Inc.	Approval:	Emilio Robledo PhD

- 3.3 Coloration test with Fluoresceín
 - 3.3.1 Sterile paper strips with fluoresceín FLU-GLO brand, or equivalent
- 3.4 Coloration test with Bengale Rose
 - 3.4.1 Sterile paper strips with bengale rose BENGALÉ ROSE brand, or equivalent
- 3.5 Coloration test with Lisamine green
 - 3.5.1 Sterile paper strips with lisamine green LISAMINE GREEN brand or equivalent
- 3.6 Distant direct ophthalmoscopy
 - 3.6.1 Dark room for evaluation
 - 3.6.2 Ophthalmoscope OTOSCO brand or equivalent

4. Responsibilities

The application and follow-through of this document should be undertaken by a licensed veterinarian or an individual with a post-graduate degree in veterinary medicine. Those undertaking this protocol can count on the support of the medical group working with ADER Enterprises, Inc. via e-mail communication at info@aderent.com

5. Procedure

- 5.1 Compilation of the patient's data.
 - 5.1.1 Fill-out Form A (attached) with the following required patient information:
 - 5.1.1.1 Breed
 - 5.1.1.2 Age
 - 5.1.1.3 Sex
 - 5.1.1.4 Weight
 - 5.1.1.5 Preliminary case history
- 5.2 Ocular inspection
 - 5.2.1 Both eyes should be inspected, despite the condition being noticed in only one eye, both should be inspected to determine:
 - 5.2.1.1 Facial symmetry
 - 5.2.1.2 Eyelid (palpebral) position
 - 5.2.1.3 Presence of ocular pain/discomfort
 - 5.2.1.4 The observations and/or results of the evaluations should be documented in the Preliminary Case History section of Form A below

 ADER Enterprises Inc. aderent.com	3 of 9	SOP #:	ESA - 001
		Revision #:	A
		Implementation Date:	12/Dec/07
Page #:		Last Reviewed/Update Date:	30/Apr /08
SOP Owner:	ADER Enterprises Inc.	Approval:	Emilio Robledo PhD

- 5.3 The patient's visual acuity is determined after carrying out the following evaluations
- 5.3.1 *Pupillary reflex evaluation:* By employing the use of a portable light source an evaluation of a pupil's reaction to light in the eye can be carried out
 - 5.3.2 *Response-to-threat evaluation:* A movement with the hand (without substantially moving air) toward the pet's eye will allow for the animal's reaction, if normal there will be a defensive closing of the eyelids and movement of the head
 - 5.3.3 *Obstacle course response evaluation:* A series of obstacles are placed in a closed room/area and the pet's owner is at the opposite end of the area. When given the signal the owner will call only once for the pet, by name, to come. The pet should accomplish this task without bumping any obstacles. This test is to be carried out in varying degrees of light/darkness
 - 5.3.4 *Response-to-cotton ball evaluation:* A cotton ball is dropped in front of the pet and the pet's behavior is observed. Normally, the pet will visually follow the fall of the cotton ball
 - 5.3.5 *Edge-of-Cliff response:* The pet is placed, under control, on the edge of a table to determine if the pet acknowledges the danger
 - 5.3.6 All observations obtained from the aforementioned evaluations should be documented in the Preliminary Case History section of the attached Form **A**

5.4 Lacrimal film of the eye is evaluated using the following tests:

- 5.4.1 *Schirmer test:* The Schirmer test allows study of total lacrimal secretion, also known as the sum of the base secretion plus the reflex secretion

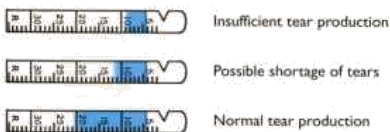
As with all Schirmer tests, test strips are employed (Whatman filter paper No. 41, 5 mm wide X 30 mm long – graduated)

The strip is placed in the external conjunctive inferior, in a low-light environment, for one minute with the patient's eyes open (refer to the following images)

 Schirmer Tear Test strip and procedure



Results



 ADER Enterprises Inc. aderent.com	ADER Enterprises Inc.	SOP #:	ESA - 001
		Revision #:	A
		Implementation Date:	12/Dec/07
Page #:	4 of 9	Last Reviewed/Update Date:	30/Apr /08
SOP Owner:	ADER Enterprises Inc.	Approval:	Emilio Robledo PhD

Schirmer Test

Normal values are realized when levels of tear production surpass 15 mm. Less than 5 mm indicates lacrimal hyposecretion.

- 5.4.2 The results obtained from the Schirmer test should be documented in the corresponding section of Form **A**, below
- 5.5 The following corneal coloring tests should be carried out:

- 5.5.1 *Fluorescein coloring:* Fluorescein consists of the chemical formula C 20 H 10 N 2 O 5.

This test is useful in identifying superficial scratches or other problems with the surface of the cornea; it is also possible to see with greater clarity the mucin layer.

The application can be accomplished using fluorescein between 1% and 2%, on impregnated dry paper strips. The paper strips should be moistened in saline solution (without preservatives) before using.

Three minutes after applying the fluorescein the investigation can be carried out using a Slit Lamp with a cobalt blue filter (Wratten Kodak 47 B filter)

- 5.5.2 *Coloration with bengale rose:* Bengale rose is an effective colorant that highlights the mucin layer.

It is precisely the mucin, or lack thereof, where the bengale rose aids in evaluating the level of protection offered in the lacrimal film to the ocular surface.

A 1% concentration is used. In many cases lower levels are employed, sometimes as low as .25%, due to the conjunctive irritation it can produce.

This conjunctive irritation is one of the principal defects of bengale rose, and it is advised that a topical anesthetic be applied to the eye prior to applying the bengale rose.

This test has been standardized as the *National Eye Institute/Dry Eye Clinical Test* applying 5 microliters of bengale rose, prior to applying a topical anesthetic, and examining the patient using a Slit Lamp with a green filter.

The level of the condition is qualified according the level of coloration and the number of affected areas.

- 5.5.3 *Coloration with lisamine green:* Lisamine green was introduced in 1973 as an effective colorant and identifies deteriorated cells and mucosal filaments present in the lacrimal film.

1% concentration is used, and is a colorant which is an artificially produced organic acid that contains two aminofenil groups like tetrazolic salts.

It will not color healthy cells and the advantage it has over bengale rose is the superior contrast in color against blood vessels and hemorrhages (green against red) and the fact that it produces virtually no burning sensation.

 ADER Enterprises Inc. aderent.com	SOP #: ESA - 001	
		Revision #: A
		Implementation Date: 12/Dec/07
Page #: 5 of 9	Last Reviewed/Update Date: 30/Apr /08	
SOP Owner: ADER Enterprises Inc.	Approval: Emilio Robledo PhD	

5.5.4 The observations obtained in completing these aforementioned coloration tests (fluorescein, bengale rose and lisamine green) should be noted in the corresponding section of the attached Form **A**

5.6 The technique described below should be employed during the Distant Direct Ophthalmoscopy

5.6.1 The room where the examination is carried out should be completely dark.

5.6.2 For this test an ophthalmoscope adjusted in the range of +1 to +2 D should be employed so as to confirm a reflection from the Tapetum Lucidum, looking into the pupil directly or slightly above the horizontal plane.

Note: For this examination it is not necessary to apply any agents for dilation of the pupils.

5.6.3 The examination should be conducted at a distance of approximately one meter as observed in the following image



Distant direct ophthalmoscopy

5.6.4 The results of this examination should be documented in the corresponding section of the attached Form **A**

 ADER Enterprises Inc. aderent.com	6 of 9	SOP #:	ESA - 001
		Revision #:	A
		Implementation Date:	12/Dec/07
Page #:		Last Reviewed/Update Date:	30/Apr /08
SOP Owner:	ADER Enterprises Inc.	Approval:	Emilio Robledo PhD

6. Form A

- 6.1 This form should be filled out completely for each veterinary patient (canine or feline) whose anterior section has been examined according to the aforementioned procedure.
- 6.2 General information of the attending veterinarian.

Date:	Address:
Name:	City:
Degree:	Phone:
School:	Email:

6.2 Patient Information

6.2.1	Breed	
6.2.2	Age	
6.2.3	Sex	
6.2.4	Weight	
6.2.5	Preliminary Case History	

 ADER Enterprises Inc. aderent.com	ADER Enterprises Inc.	SOP #:	ESA - 001
		Revision #:	A
		Implementation Date:	12/Dec/07
Page #:	7 of 9	Last Reviewed/Update Date:	30/Apr /08
SOP Owner:	ADER Enterprises Inc.	Approval:	Emilio Robledo PhD

6.3 Ocular examination

6.3.1 Document your observations during the ocular evaluation

6.3.1.1	Facial symmetry	
6.3.1.2	Eyelid (palpebral) position	
6.3.1.3	Demonstration of ocular discomfort	

Note: Refer to section 5.2 of this procedure

6.4 Visual acuity

6.4.1 Note your observations encountered during examination of the patient in response to the following visual acuity tests

6.4.1.1	Test: pupillary reflex	
6.4.1.2	Test: reponse to threat	
6.4.1.3	Test: obstacle course	
6.4.1.4	Test: response to cotton ball	

Note: Refer to section 5.3 of this procedure

 ADER Enterprises Inc. aderent.com	SOP #: ESA - 001	
		Revision #: A
		Implementation Date: 12/Dec/07
Page #: 8 of 9	Last Reviewed/Update Date: 30/Apr /08	
SOP Owner: ADER Enterprises Inc.	Approval: Emilio Robledo PhD	

6.5 Lacrimal test (Schirmer test)

6.5.1 Document the obtained measurements and observations from the Schirmer test

6.5.1.1	Right Eye	Results: Observations:
6.5.1.2	Left Eye	Results: Observations:

Note: Refer to section 5.4 of this procedure

6.6 Corneal colorations tests

6.6.1 Document the results from the various colorations

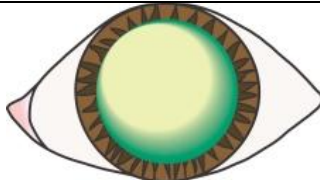
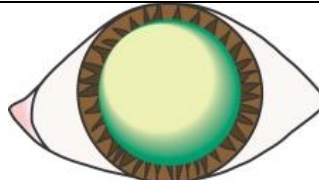


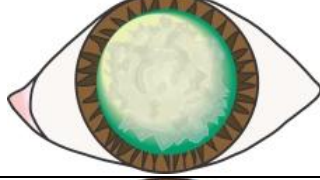
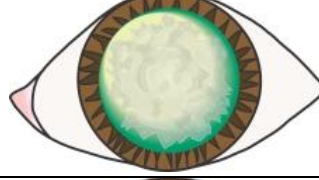
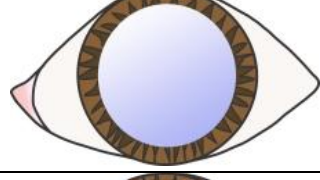
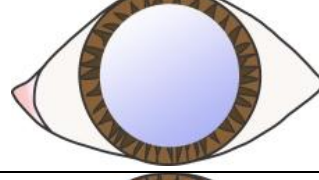
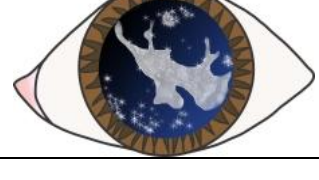

6.6.1.1	Fluorescein test	Right eye: Left Eye:
6.6.1.2	Bengale Rose test	Right eye: Left Eye:
6.6.1.3	Lisamine Green test	Right eye: Left Eye:

Note: Refer to section 5.5 of this procedure

 ADER Enterprises Inc. aderent.com	9 of 9	SOP #:	ESA - 001
		Revision #:	A
		Implementation Date:	12/Dec/07
Page #:		Last Reviewed/Update Date:	30/Apr /08
SOP Owner:	ADER Enterprises Inc.	Approval:	Emilio Robledo PhD

6.7 Evaluation of the transparency of the lens (cataract detection)

6.7.1 Document the results observed during the evaluation of the lenses, indicating with a check mark the box that corresponds to the level of cataract development encountered

	Right Eye	Left Eye
6.7.1.1 Without cataract		
6.7.1.2 Incipient Cataract		
6.7.1.3 Immature Cataract		
6.7.1.4 Mature Cataract		
6.7.1.5 Hyper-Mature Cataract		

Note: Refer to section 5.6 of this procedure

Final Comments: _____

