

<p style="text-align:center">SJÖGREN'S INTERNATIONAL COLLABORATIVE CLINICAL ALLIANCE (SICCA) CHAPTER 9 OCULAR EXAMINATION STANDARD OPERATING PROCEDURE (SOP)</p>

****When the SICCA ocular exam is scheduled the participant should be instructed to discontinue the use of eye drops (including Restasis) for 12 hours prior to the exam.**

SICCA ocular exam order:

- A) Tear Film Osmolarity
- B) Schirmer Test
- C) Ocular Staining with Fluorescein
 - Tear Break Up Time
 - Measurement the cornea AND conjunctiva
- D) External Eye examination
- E) Ocular Staining with Lissamine Green
 - Measurement of the cornea AND conjunctiva
- F) RNA Conjunctival Imprint

A. Tear Film Osmolarity Test

- 1) Please place the Tearlab™ instrument, Test Cards and Control Solutions in the exam area where the testing will be performed, the night prior to the testing. The TearLab instrument must be turned on the night prior to ensure that the batteries in the Pens are fully charged. Care must be taken to ensure that no external heating or cooling source is affecting the TearLab instrument, Test Cards or Control Solutions, and that all components are at ambient temperature for at least 30 minutes prior to testing. Therefore all components must be placed in close proximity to each other. Once all components have reached ambient temperature, ensure that the device quality control procedures have been performed and passed, including testing with the Electronic Check Cards, and the “high” as well as the “normal” TearLab Control Solutions . For further information related to the operation of the machine, please refer to the manual that is available online at http://www.tearlab.com/pdfs/technical/92321_TearLab_UserMan_ENGLISH.indd.pdf
- 2) Remove either Pen from the Reader. The LCD will display “Ready.” DO NOT collect tear samples if the Reader does not display “Ready.”
- 3) Remove a Test Card from its package and attach it onto the Pen. The Pen will beep and the green light will illuminate when the Card is attached properly. The green light will remain on until tears are collected or the Pen times out (after two minutes). Remove the protective cover by holding the wings of the Test Card firmly and pulling the sheath up and off of the Test Card. Do not use a Test Card without a protective cover as it might have been used previously.

- 4) Seat the patient with chin tilted upward and eyes directed toward the ceiling. Place one hand on the face for stabilization. Do not pull the eyelid down or away from the eye. Position the tip of the Pen just above the lower eyelid. Gently lower the Pen until the bottom of the tip touches the thin line of moisture between the eyelid and eye. It is not necessary to press inward toward the eye. The Pen will beep and the green light will turn off after a successful tear collection. If you do not get an immediate beep, do NOT withdraw the Pen from the eye, but apply additional DOWNWARD pressure onto the eyelid without removing the Pen from the eyelid. (Remember, always avoid contact with the globe during collection, so apply additional pressure downward on the inner margin of the eyelid where the tear collection is to occur, and continue to avoid touching the globe itself). If the Pen still does not beep, elicit a short 1-2 mm lateral brushing motion across the eyelid - again without removing the Pen from the eyelid. This procedure will usually result in a successful collection indicated by an audio "beep" from the Pen and the green Pen light going off.

A "Below Range" message may occur due to a difficult tear collection from a patient with a very low tear meniscus and/or dry eye as may be expected in a Sjogren patient. A "Below Range" message usually occurs when the capillary channel in the test card during a tear collection is partially filled as a result of "pecking" at the eye, that is, attempting a tear collection, not getting an immediate beep from the Pen, withdrawing the Pen and then re-entering the tear lake for another attempt- resulting in two partial fills within the capillary channel with an air gap in between. If you get a "Below Range" message perform another test with a new Test Card using the above described collection technique. If a "Below Range" message persists, run a TearLab Control Solution test to confirm the accuracy of the Test Cards, and if the Test Cards are not accurate contact TearLab customer support.

Additional information on proper tear collection technique and quality control procedures can be found in the Training Video section of our website (<http://www.tearlab.com/products/doctors/videos.htm>).

- 5) Locate the code on top of the Test Card. Dock the Pen into the Reader within 40 seconds of collecting the sample. Immediately press the RECALL Key below the up or down arrows to select the Test Card code. If a code is not selected within eight seconds, the Reader will automatically use the default code displayed on the LCD. It is important to select the correct code to obtain an accurate osmolarity test result.
- 6) Press the OK Key or wait eight seconds to accept the code. The test result will display in a few seconds. Record the date and test result in the patient chart. Remove the used Test Card from the Pen by pressing forward on top of the Test Card with your thumb. Do not pull from the wings. Dispose of the Test Card.

To see a video of the procedure please go to <http://www.tearlab.com/>. Click on "See The TearLab Test" on the left hand side of the page, in the middle section.

B) The Schirmer Test

The Schirmer I test (un-anesthetized) will be performed. It must be done **before** any other tests, are performed (except for the Osmolarity test), and **before** any drops have been instilled into the eye.

- It is important that the lids are not manipulated before the test.
- The standardized Schirmer strips should be bent at the notch on the curved end of the strip and the folded end placed carefully over the lower lid margin between the junction of the middle and outer third of the lower lid, as far toward the temporal angle of the lids as possible. Every effort should be made to place the Schirmer strip over the lid margin without traumatizing the conjunctival or corneal epithelium. The Schirmer strips are labeled “R” and “L” for convenience.
- As soon as both Schirmer strips are in place, the time should be noted and the strips allowed to remain in place for a maximum of five minutes, or until the strips are completely saturated with tears, if sooner.
- The patient should keep their eyelids closed during the Schirmer test.
- After 5 minutes, or when the strips are completely saturated, carefully remove the strips.
- The amount of wetting of the strips is indicated by the blue dye impregnated in the Schirmer strip, and the measurement is made using the millimeter scale printed on each strip to note the leading edge of the dye. If the strips are not saturated in 5 minutes, enter the amount of wetting in mm in item 1 of the Eye Examination Form.
- If complete saturation of the strips occurs before five minutes, the amount of time should be recorded in item 1a.
- The strips should then be removed and 0.5% fluorescein instilled in both eyes immediately before processing the Schirmer strips. Processing the strips should take no longer than 2 minutes. During this time the fluorescein stains the patient’s tear film and any excess volume of the dye drains out of the eye through the superior and inferior puncta allowing the tear film to stabilize.
- During this two minute period each Schirmer strip is cut in half, lengthwise, using scissors that have been previously cleaned with alcohol (it is not necessary to wear sterile gloves since these specimens are not being collected for DNA or RNA analysis). The two bisected portions of each strip are placed together in separate vials, marked right and left, and then item 2 is answered.
- The two vials, which are provided by the study to collect the tear samples of the patients, are labeled with the participant ID number, held on ice, and then frozen as soon as possible at minus 80 degrees centigrade and stored for later transfer to the SICCA tissue bank in San Francisco.

C) Assessing Ocular Staining with Fluorescein at the Slit Lamp

The slit lamp should be used with 10x magnification and the illumination set on “high” for the exam. Fluorescein stains defects in the corneal epithelium that can be seen at the slit lamp using a cobalt blue filter over the light source. The pre-corneal tear film is also stained with fluorescein, allowing kinetic changes in the thickness and dispersion of the film to be observed directly so that the tear break-up time can be determined. Fluorescein

also reveals punctate epithelial erosions that may be present on the cornea. The BUT measures stability of the tear film and indicates whether the mucus component of the tear film is of normal quality or is present in normal amounts.

Measuring Tear Break-Up Time (BUT)

- Fluorescein has already been instilled in each eye from a sterile dropper bottle (containing 1 ml of 0.5% dye). This will be discarded after being opened and used for one or more patients in the same clinic. Because the solutions in the bottles do not contain preservatives, all bottles must be discarded the day they are opened **without exception**.
- One drop of 0.5% fluorescein dye was applied to the conjunctival fornix of each eye and the patient told to squeeze the eyes tightly to blink out the excess dye that is gently blotted away from the skin of the lids with a tissue.
- The interval of two minutes that is allowed to elapse before the eye is examined at the slit lamp is used to process the Schirmer strips. This allows the fluorescein to diffuse and stain the precorneal tear film and tear meniscus, and permits any excess dye to drain out through the puncta before the BUT is measured.
- Each eye is then examined at the biomicroscope (slit lamp) and the BUT is determined by asking the patient to blink once and hold their eyes open. The BUT is the time in seconds between the patient's last blink and the first appearance of a random dry spot on the cornea. The eyelids should not be held open or the eyes manipulated in any fashion.
- Care should be taken that the examining room does not have a draft or a forced air unit that might prematurely dry out the tear film.
- The endpoint is the time in seconds that it takes for the first blue dry spot to appear on the cornea while observing the greenish-yellow tear film with the cobalt filter on the slit lamp. Three consecutive readings should be taken for each eye and the mean value recorded. Concurrent observation of an easily visible second hand or stopwatch is necessary to insure repeatable measurements of the BUT.

- A BUT of 10 seconds or more is normal and is noted on item 17. Less than 10 seconds implies a mucus deficiency, or a combined deficiency of both the aqueous and mucus components of the tear film and (note seconds in item 17a). In patients with moderate to severe clinical KCS, the BUT may be almost immediate, with multiple dry spots present on the cornea after each blink.
- The presence or absence of mucus shreds and /or debris in the tear film should also be noted (item 18).
- Measurement of the BUT should take no longer than two minutes so that the next phase of the examination will be performed at an interval of no longer than four minutes after the fluorescein is instilled. Because the staining of the corneal epithelium is a dynamic, time-sensitive, process, it is important that the grading of the fluorescein staining pattern in the cornea is always initiated at the same time following fluorescein dye instillation. Grading the corneal fluorescein staining pattern before four minutes may result in a much lower SICCA score. Grading the fluorescein staining pattern after the dye has been allowed to diffuse for 8 to 10 minutes tends to result in more confluent staining pattern and leads to a falsely high SICCA score.

Measuring Punctate Epithelial Erosions (PEE) on the Corneas

- The cornea should be examined with the slit lamp continuing to use the cobalt blue filter. Any PEE that stain with fluorescein should be noted, counted, and given a score. If 1-5 PEE are seen, the corneal score is 1; if 6-30 PEE are seen, the score is 2; if >30 PEE are seen, the score is 3.
- If PEE occur in the visual axis (central 4 mm of the cornea) another point is added. If one or more corneal filaments are seen anywhere on the cornea, one more point is added. If one or more patches of confluent staining are seen anywhere on the cornea, another point is added. Enter any extra points that apply in item 20. Then enter the total fluorescein score for the cornea (PEE grade plus any extra points) in the central square in item 19. The maximum cornea score is 6.
- This scoring is based on central PEE, filaments, or confluent staining being associated with significantly worse clinical disease.

D. The External Eye Examination at the Slit Lamp

The external eye examination is performed after the Schirmer test and the assessment of ocular staining with fluorescein has been completed. The slit lamp should be used for the examination, but magnification and illumination may be adjusted according to the needs of the examining ophthalmologist and the comfort of the patient. This portion of the eye examination is not time dependent, because ocular staining characteristics are not being measured. Therefore, the external exam should be performed carefully, **noting the presence or absence of lid abnormalities (yes/no) in items 3-6; conjunctival abnormalities in items 7-8; any confounding diseases in items 9-10; the presence of punctal occlusion in items 11 and 12; and corneal abnormalities in items 13-16.**

E. Assessing Ocular Staining with Lissamine Green at the Slit Lamp

Lissamine green is a vital dye that, like fluorescein, stains corneal and conjunctival epithelial defects, but its main advantage lies in the fact that it stains keratinized and devitalized epithelial cells, goblet cells, mucus, and epithelial filaments. The staining pattern of lissamine green is best seen by using 10x magnification and a neutral density filter over the slit lamp light source without a color filter. Ocular staining with lissamine green should be performed following the external eye exam.

- Lissamine green dye is instilled in each eye from sterile dropper bottles (containing 1 ml of 1% dye). As with fluorescein, the lissamine green solution does not contain a preservative and must be discarded the same day it is opened **without exception**. Unlike Rose bengal, lissamine green is not irritating to the eye so it can be applied without needing to use topical anesthetic. A full drop of the dye can be used without causing pain.
- A full drop of lissamine green is applied to the inferior fornices of both eyes of the patient who is then instructed to squeeze their eyes closed tightly. Any excess lissamine green is wiped from the skin of the lids with a tissue.
- After the dye is applied, the eyes are observed at the slit lamp using 10x magnification with white illumination through a neutral density filter. Unlike the time lapse necessary to achieve maximum staining with fluorescein, **the patient must be examined immediately after instilling lissamine green dye because the intensity and extent of the ocular staining diminishes rapidly after the first two minutes, especially in patients with mild to moderate KCS**. Failure to exam the eyes immediately after instillation of lissamine green may lead to a falsely low ocular SICCA score. If adequate dye was not initially instilled, or the examiner allowed too much time to elapse before determining the score, a second drop of the dye may be instilled and the examination performed again immediately. The patient should be instructed to blink several times to continue to remove any excess dye and to eliminate dye that is pooling in the conjunctival folds but that is not actually staining involved epithelial cells and goblet cells.
- Because the Schirmer strip often abrades the temporal bulbar conjunctiva, resulting in false positive lissamine green staining, blotchy staining that exactly matches the placement of the Schirmer strip should be ignored.
- The nasal and temporal bulbar conjunctiva in the inter-palpebral area should be graded separately. The grading of the conjunctival lissamine green staining pattern is performed using a modification of the Oxford grading scheme that was described by Bron, et al. For the purposes of our grading system, grade 0 will consist of 0 to 9 dots of lissamine green staining on the bulbar conjunctiva (nasal and temporal bulbar conjunctiva graded separately), grade 1 is 10 to 32 dots, grade 2 is 33-100 dots, and grade 3 is greater than 100 dots. The nasal and temporal inter-palpebral bulbar conjunctiva can each have a score of no more than 3 with a maximum conjunctival score of 6. (Enter scores in the two outer squares in item 19.)
- The total ocular SICCA score is a summation of the fluorescein score for the cornea and the lissamine green scores for the nasal and temporal bulbar conjunctiva. The maximum SICCA corneal-conjunctival score for any eye is 12.

The eyes are graded and scored separately. The scores of the two eyes are not added together. Enter the total ocular SICCA score for each eye in item 21.

Addendum January 2012. We would like to now grade BOTH the conjunctiva and the cornea with lissamine AND fluorescein. Cornea will be graded with both fluorescein and lissamine green, using the original cornea grading scale (0, 1-5, 6-30, >30) and bonus points for the cornea (patches of confluent staining, staining in the pupillary area, one or more filaments). Conjunctiva will continue to be graded with the original conjunctival grading scale (0-9, 10-32, 33-100, >100).

To facilitate conjunctival grading with fluorescein, cover the slit lamp illumination with a yellow filter (w/o blocking your view). The recommended filter is: Kodak 3 x 3", #12 Deep Yellow Optical Wratten 2 Filter.

The original eye form is unchanged—please continue to grade cornea with fluorescein and conjunctiva with lissamine green, and fill out the form as you have been doing.

We have added an additional page to record the new grades. Here, please record your grades of the conjunctiva with fluorescein and the cornea with lissamine green. Note that the grading system is identical, just with the different stain. We understand that now you have to record fluorescein cornea on one form, and fluorescein conjunctiva on another. However, we felt that it was important to keep the original eye exam exactly as it has always been.

F. RNA Conjunctival imprint (USA, Denmark, Argentina and United Kingdom sites only)

The procedure for collecting RNA by conjunctival imprint is based on the same method used for conjunctival impression cytology. Mixed cellulose ester membranes are used to collect cells from the conjunctival surface for RNA isolation. A supply of MF-Millipore mixed cellulose ester membranes 13 millimeter in diameter (Millipore 0.45 micron HA, Cat No. HAW P01300) will be supplied to each of the participating centers, as well as several sets of RNase-free surgical eye forceps without teeth, and Sarstedt cryovials (or equivalent cryovials) for the specimens, each containing RNAlater® reagent. Cryovials must be filled to near capacity with the RNAlater® reagent to ensure constant immersion and preservation of the collected samples. The RNA conjunctival imprint specimen is taken after all the other diagnostic ocular tests have been performed and each eye has been given an ocular SICCA score. **The investigator should put on sterile gloves before the following procedures are performed.**

- First one of the 13 millimeter diameter white membranes is cut in half using sterile technique (sterile scissors and forceps, and sterile gloves) to avoid RNA contamination. The membranes are packaged with blue separators between them.
- After topical anesthesia with a drop of proparacaine, the patient's eyelid is held open for 10 seconds to allow the ocular surface to dry, and then one half of a MF-Millipore mixed cellulose ester membrane is grasped with a the sterile RNase-free

- toothless forceps and placed gently against the temporal bulbar conjunctiva in the interpalpebral area at least 2 millimeters from the temporal limbus. It is held against the temporal bulbar conjunctiva for 10 seconds.
- The closed forceps tips are used to press the membrane against the conjunctiva, and the surface of the membrane is massaged with the closed forceps moving back and forth to insure that it sticks to the conjunctival surface.
 - The edge of the millipore membrane is then carefully grasped with the toothless forceps and the membrane is slowly peeled away from the conjunctiva, removing the superficial epithelial surface that adheres to the membrane. The conjunctiva should stick to the membrane as if it were scotch tape, the conjunctival surface adhering to the membrane so tightly that it stretches as it is slowly peeled off.
 - The membrane is then placed in the provided cryovial containing of RNeasy® reagent.
 - The cryovial is labeled with the patient's number and marked right or left eye.
 - The same procedure is then repeated on the temporal bulbar conjunctiva of the other eye using the other half of the membrane, placing the specimen in a separate cryovial, and labeling it appropriately.
 - The cryovials should be placed on ice as soon as the specimens are collected and then transported to the laboratory where they are left at 4°C for 24 hours. The next day the cryovials are transferred to storage at -80°C until they are ready for RNA extraction to be performed.
 - Total time for the eye examination should take no longer than 20 to 25 minutes.

Supplies and Equipment Needed for Exam

- Slit Lamp
- Cryovials labeled with SICCA Participant ID
- RNAase-free surgical eye forceps (for Argentina, Denmark, UCSF and United Kingdom)
- *Schirmer Strips with color bar by Eaglevision (Cat # 0039)
- *1% Lissamine Green – Leiter's Pharmacy
- *0.5% Fluorescein – Leiter's Pharmacy
- *RNeasy® by Ambion (Cat # 7020 100ml Or 7021 for 500 ml) for (Argentina, Denmark, United Kingdom and UCSF)
- *MF- Millipore Filter mixed cellulose ester membranes, .45µm, 13 mm in diameter for Argentina, Denmark, United Kingdom and UCSF). Cat # HA P01300

** Contact Yvonne De Souza at yvonne.desouza@ucsf.edu for these supplies.