

**STANDARD OPERATING PROCEDURE (SOP):  
EYE EXAMINATION FORM**

**Schirmer Test**

The Schirmer I test (un-anesthetized) will be performed. It must be done **before** any other tests are performed 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.
- As soon as both Schirmer strips are in place, the time must 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 measured in millimeters from the notch to the line of wetting. This is done with the millimeter scale on the package containing the Schirmer strips, or if the strips are marked in millimeters, measurements are made directly on the strips. If the strips were 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, that amount of time should be recorded in item 1a.
- Each Schirmer strip is then 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 analysis). The two bisected portions of each strip are placed together in separate vials, marked right and left and then item 2 is answered.
- These two vials, which are provided by the study to collect the tear samples of the patients, are then labeled with the participant ID number and immediately frozen at minus 70 degrees centigrade and stored for later transfer to the SICCA tissue bank in San Francisco.

### **Slit lamp Examination**

The slit lamp examination will be performed after the Schirmer test and prior to instillation of any drops. The slit lamp should be used with 10x magnification and the illumination set on "high" for all exams.

- The presence or absence of lid abnormalities will be noted (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.

### **Ocular Staining with Fluorescein**

Fluorescein stains defects in the corneal and conjunctival 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 is instilled in each eye from sterile dropper bottles (containing 1 ml of 0.5% dye). These 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, they must be discarded the day they are opened **without exception**.
- One drop of 0.5% fluorescein dye is 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.
- An interval of 60 seconds should then be allowed to elapse before the eye is examined at the slit lamp, allowing the fluorescein to diffuse and stain the precorneal tear film and tear meniscus.
- 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).
- **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.

### **Ocular Staining with Lissamine Green**

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 a neutral density filter over the slit lamp light source.

- 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 the neutral density filter. The patient can be examined immediately, unlike the time lapse necessary with fluorescein.

- 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 must be ignored.
- The nasal and temporal bulbar conjunctiva in the inter-palpebral area will 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.

### **RNA Conjunctival imprint (USA and Denmark sites only)**

The procedure for collecting RNA by conjunctival imprint is based on the same method used for conjunctival impression cytology. A supply of MF-Millipore nitrocellulose membranes (Millipore, #VSWPO1300, 0.025 micron, 13 millimeter diameter) will be supplied to each of the participating centers, as well as several sets of surgical eye forceps without teeth, and Sarstedt cryovials (or equivalent cryovials) for the specimens, each containing 350 micro liters of Trizol reagent. 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.

- First cut one of the 13 millimeter diameter membranes in half using sterile technique (sterile scissors and sterile gloves) to avoid RNA contamination.
- After topical anesthesia with proparacaine, and holding the eyelid open for twenty seconds to allow the ocular surface to dry, use the sterile toothless forceps to place one half of the membrane (dull side down) gently against the temporal bulbar conjunctiva in the interpalpebral area at least 2 millimeters from the temporal limbus.
- The closed forceps tips are then used to press the membrane against the conjunctiva, and the surface of the membrane is massaged with the closed forceps running 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 gently peeled off the conjunctiva, removing the superficial epithelial surface that adheres to the membrane.
- The membrane is placed in the provided cryovial containing 350 micro liters of Trizol 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 they should then be transported to the laboratory as soon as possible.
- In the laboratory the cryovials are vortexed for approximately thirty seconds and then frozen at  $-70$  degrees centigrade and stored.
- Later the RNA is extracted from the membranes prior to evaluation of gene expression by PCR or microarray.