

Grading Of Corneal and Conjunctival Staining in the Context of Other Dry Eye Tests

Anthony J. Bron, FCOphth, FMedSci, Victoria E. Evans, BOptom(Hons), and Janine A. Smith, MD

Abstract

Purpose:

To describe the Oxford Scheme for grading ocular surface staining in dry eye and to discuss optimization of stain detection using various dyes and filters. Also, to propose a sequence of testing for dry eye diagnosis.

Methods:

The grading of corneal and conjunctival staining is described, using the Oxford Scheme, including biomicroscopy, optical filters, illumination conditions, and the characteristics of and instillation techniques used for, selected clinical dyes.

Results:

A series of panels, labeled A–E, in order of increasing severity, reproducing the staining patterns encountered in dry eye, are used as a guide to grade the degree of staining seen in the patient. The amount of staining seen in each panel, represented by punctate dots, increases by 0.5 of the log of the number of dots between panels B to E. The use of the vital dyes fluorescein, lissamine green, and rose Bengal is described; fluorescein and lissamine green, used in conjunction with appropriate absorption filters, are recommended for use in clinical trials. The placement of staining in relation to the sequence of other diagnostic tests is discussed.

Conclusions:

The monitoring and assessment of corneal and conjunctival staining can be greatly enhanced by the use of a grading scale, controlled instillation of dyes, and standard evaluation techniques. This is of particular benefit in clinical trials, where ocular surface staining is commonly employed as an outcome measure

Key Words: grading, cornea, conjunctiva, staining, fluorescein, rose Bengal, lissamine green, filter, clinical trial

(*Cornea* 2003;22:640–650)

Grading staining of the ocular surface after instillation of vital dyes is a critical component of dry eye diagnosis. Pflüger was the first to describe vital staining of the cor-

nea and conjunctiva in 1882,¹ and Fromm and Groenouw (cited by Joyce) reported the use of fluorescein to stain damaged corneal epithelium in 1891.² Staining of the ocular surface with various dyes is used to characterize disease, assess its severity, and monitor the clinical response to therapy. It is also used as an outcome measure in clinical trials. For any of these purposes, but particularly for clinical trials, it is essential to standardize the procedures used. This paper describes a standardized approach to the grading of ocular surface dye staining in dry eye, using the Oxford grading system.^{3,4} Much of the paper deals with the details of dye selection and instillation. The approaches proposed are equally relevant to other grading systems.^{5–7}

Because of the invasive nature of some of the tests performed in the diagnosis of dry eye, it is important to perform them in a sequence that minimizes their interaction and limits the extent to which one test influences the outcome of the tests that follow. The sequence described here assumes that most or all tests are performed at the same visit for reasons of convenience and, in the clinical trial setting, to achieve compliance. However, instances may arise when, for operational reasons, tests would be conducted at separate clinic visits.

THE GRADING SCHEME

The Oxford grading scheme was developed to quantify the amount of epithelial surface damage in patients with dry eye. Because the grading charts used were devised to represent patterns of staining commonly encountered in dry eye, its use is not recom-

Received for publication
January 28, 2003;
accepted June 17, 2003.

From the Nuffield Laboratory of Ophthalmology (Drs Bron and Evans), University of Oxford, Oxford, UK; and the Division of Epidemiology and Clinical Research (Dr Smith), National Eye Institute, National Institutes of Health, Bethesda, MD.

Reprints: Anthony J. Bron, Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW, England (e-mail: enquiries@eye.ox.ac.uk).

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mended to quantify staining in other ocular surface disorders.

The Oxford system uses a chart consisting of a series of panels, labeled A-E in order of increasing severity. In each chart, staining is represented by punctate dots. The number of dots increases by 1 log unit between panel A and B and by 0.5 log units between each of panels B to E inclusive. To grade staining, comparisons are made between the panels and the appearance of staining on the exposed interpalpebral conjunctiva and cornea of the patient.

The details of the chart are presented in (Fig. 1) and are resupplied in Figure 4 in a simplified form suitable for use in clinical trials. The charts presented are from a high-resolution scanned image of the original chart devised in 1984. In Figure 1, each panel indicates the dot count on a log scale. This information has been removed from the chart presented in Figure 4, and it is suggested that potential users scan in and print out the sheet for clinical use.

METHOD FOR USING THE GRADING SCHEME

After instillation of the dye, the eye is examined with the slit-lamp microscope using standard settings (eg, $\times 16$ magnification with $\times 10$ oculars using the Haag-Streit slit-lamp). For clinical trial purposes it is essential that all observers use exactly the same tech-

nique, including slit-lamp magnification and illumination. If this cannot be achieved, then it is at least advisable that the same microscope be used within a unit, from visit to visit.

To observe and grade staining over the entire corneal surface, it is important for the examiner to raise the subject's upper eyelid slightly (Fig. 2b). For the conjunctiva, the temporal, interpalpebral conjunctival zone is observed with the subject looking nasally along the horizontal plane, and the nasal conjunctiva is observed with the subject looking temporally (Figs. 2a and 2c, respectively). The system can also be adapted to include the upper and lower conjunctiva for some purposes. (Fig. 3) shows lissamine green (Fig. 3a) and fluorescein (Fig. 3b) staining of the upper bulbar conjunctiva of the same eye.

When an assessment is made, it is important that the gestalt appearances of the ocular staining and panels are compared. The grading panels should be readily visible to the examiner during the evaluation. It is convenient to post the panel on a nearby surface that allows the examiner to view it while looking up from the slit-lamp microscope. The examiner can then look back at the patient and easily compare the overall appearance of the corneal or conjunctival staining. No attempt should be made to count the dots or to assess the position or confluence of the dots. The best way to approach this is for the observer to see which panel best represents the number of dots on the cornea or the conjunctiva and record the corresponding grade number. With this approach, selecting the appropriate grade is surprisingly easy.

The verbal descriptor is not used other than as a guide to the relative severity of staining in each grade; only the grade number is recorded.

SELECTION OF STAINS FOR ASSESSING OCULAR SURFACE DAMAGE

Different vital dyes may be selected for staining the ocular surface, each with relative

PANEL	GRADE	CRITERIA	DOT COUNT	LOG	VERBAL DESCRIPTOR
A	0	Equal to or less than panel A	1	0	Absent
B	I	Equal to or less than panel B, greater than A	10	1.0	Minimal
C	II	Equal to or less than panel C, greater than B	32	1.5	Mild
D	III	Equal to or less than panel D, greater than C	100	2.0	Moderate
E	IV	Equal to or less than panel E, greater than D	316	2.5	Marked
>E	V	Greater than panel E	>316	>2.5	Severe

FIGURE 1. This figure depicts the grading panels, their numerical grade, the dot count and log of dot numbers, as well as the verbal descriptor for each grade.

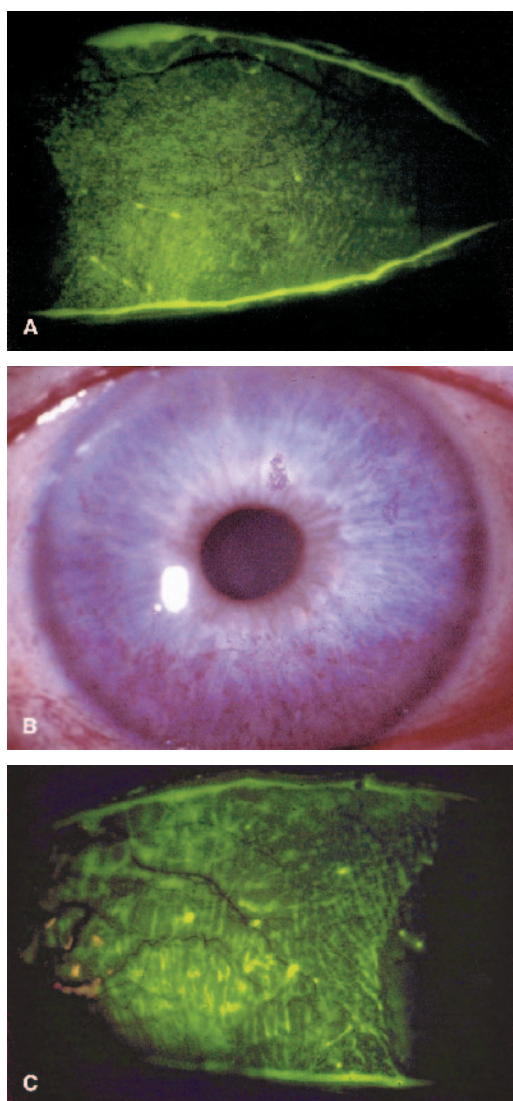


FIGURE 2. Eye positions adopted for grading. a, The nasal conjunctiva of the right eye is observed with the subject looking temporally in the horizontal plane. In this example, punctate fluorescein staining is evident. b, Assessment of corneal staining is performed with the upper lid raised. The cornea shown here has been stained with rose Bengal. Staining is seen well against a blue iris. c, Temporal conjunctival staining of the right eye is observed with the subject looking nasally along the horizontal plane. Fluorescein pooling is seen, but no staining. The coarse spots of fluorescence are stained mucus. Fluorescein staining photographed using a narrow-band blue exciter filter and a yellow Kodak Wratten 12 barrier filter.

advantages and disadvantages. Lists of manufacturers of dyes and filters used to assess staining can be found in (Tables 1 and 2).

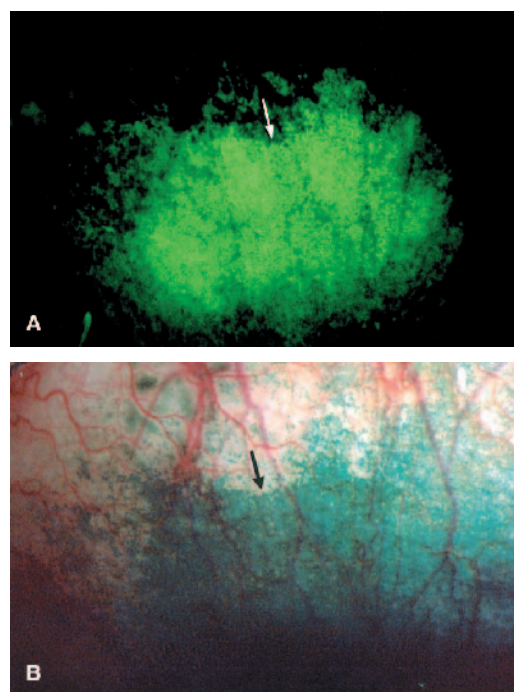


FIGURE 3. The grading system can be adapted to include the upper and lower conjunctiva for some purposes. In this example the upper conjunctiva of the same eye has been stained sequentially with (a) fluorescein (narrow-band blue exciter filter; yellow Kodak Wratten 12 barrier filter) and (b) lissamine green. Corresponding regions of staining are indicated with arrows.

The cornea and conjunctiva are observed after instillation of fluorescein, rose Bengal, or lissamine green. Other dyes, not commercially available, have also been recommended.^{8,9} Fluorescein sodium is mostly ionized at physiologic pH and does not penetrate the intact corneal tissue or stain vital tissue.¹⁰ It reaches its maximum fluorescence at pH 8.0. In the presence of an epithelial defect, staining must be graded as quickly as possible after instillation because the dye then diffuses rapidly into the tissue, and its high luminosity results in a blurring of the staining margin.

Staining after the instillation of rose Bengal or lissamine green persists for longer at high contrast and may therefore be observed for a considerable period after instillation. This is convenient for both grading and photography.

TABLE 1. Product Information for Ocular Surface Staining: Dyes

Product Dyes	Company	Type	Specifications	Units
Minims® fluorescein sodium	Chauvin Pharmaceuticals, Essex, UK	Solution	2%	20 × 40 µL unit dose
Fluorets®	Chauvin Pharmaceuticals, Essex, UK or Akorn, IL	Strips	1 mg	100 sterile strips
Ful-Glo®	Akorn, IL	Strips	0.6 mg	300 strips
Rose Bengal	Akorn, IL	Strips	1.3 mg	100 sterile strips
Minims® Rose Bengal	Chauvin Pharmaceuticals, Essex, UK	Solution	1.0%	20 × 40 µL unit dose
Lissamine green	Akorn, IL Rose Stone Enterprises, Alta Loma, CA	Strips	1.5 mg	100 sterile strips
Lissamine green	Leiters Park Aveune Pharmacy, CA	Solution	1.0%	As ordered

FLUORESCEIN SODIUM

Fluorescein has many advantages over other dyes, including its commercial availability and high tolerability. Single applications of fluorescein do not cause stinging. Although fluorescein has been reported to induce ocular surface staining after sequential instillations of highly concentrated solutions in normal subjects, this is not relevant to its use as described in this article.¹¹

Fluorescein sodium is available in a 2% solution in unit-dose form. Sterile, preserva-

tive-free unit-dose preparations avoid the risk of bacterial contamination associated with multidose preparations. Fluorescein sodium is also available in the form of paper strips with fluorescein-impregnated tips. Saline is used to wet the strip and deliver diluted fluorescein to the ocular surface.

For both the drop and paper strip methods of instillation, the aim is to achieve highly fluorescent staining of areas of loss of epithelial integrity. At a 2.0% concentration, fluorescein sodium is nonfluorescent, whereas

TABLE 2. Product Information for Ocular Surface Staining: Filters

Product Filters	Company	Type	Specifications	Units
Wratten 47 blue excitation filter	Kodak, distributed by Edmund Industrial Optics, NJ	Gelatin	Transmission peak 410–500 nm	7.5 × 7.5 cm
Wratten 12 yellow absorption filters	Kodak	Gelatin	Transmission peak 510+ nm	7.5 × 7.5 cm
Yellow interference filter	Edmund Industrial Optics, NJ	Glass filter	520 nm	7.5 × 7.5 cm
Wratten 58 absorption filter	Kodak		Transmits in the green	
Wratten 92 absorption filter	Kodak 92	Gelatin	Transmits in the red	7.5 × 7.5 cm
Hoya 25A absorption filter	Hoya	Glass	Transmits in the red	6.2 cm diameter

fluorescein is highly fluorescent at concentrations in the region of 0.1%.¹⁰ In this concentration range, the luminosity of fluorescence is proportional to the tear film thickness observed.

Instillation Technique

Quantified Drop Instillation

For clinical trials, a specific volume and mode of administration must be used. A small drop of 2% sterile fluorescein can be instilled into each conjunctival sac with a micropipette (using a sterile tip) or a glass capillary tube. The volume instilled is usually about 2 μ L. Using larger volumes at 2% concentration risks the possibility that in a very dry eye, there will be insufficient tears to dilute the instilled fluorescein into the fluorescent range. At a higher concentration fluorescence is quenched, and the fluorescein remains dark in appearance.

Unquantified Instillation—Impregnated Paper Strips

This is a convenient approach in the clinic using the following method of application:

A single drop of sterile, unit-dose saline is instilled onto a fluorescein-impregnated strip. The drop is allowed to just saturate the impregnated tip, at which point the excess is immediately shaken free into a waste bin. The lower eyelid of the right eye is then pulled downward, and the strip is immediately tapped onto the lower tarsal conjunctival surface. A similar procedure is carried out in the left eye. The tap must be rapid enough to deliver only a tiny volume, and some experience is required. If too large a volume is delivered, then the concentration in the tear film may be too high, and the tear film and staining pattern will be nonfluorescent.

Timing

Because fluorescein diffuses rapidly into tissues in the presence of an epithelial defect, the delineation of punctate staining is lost in a short period of time. It is therefore

essential to assess staining rapidly in the right and then the left eye in sequence, so that the staining pattern observed in the second eye is as crisp as that seen in the first eye. This is worthwhile because, in clinical studies, the fluorescein breakup time (FBUT) is usually performed before grading of the staining.

If it is of interest to photograph the staining pattern, then instillations should be separated in time, and photography should follow immediately after each instillation.

Exciter and Barrier Filters

The correct way to assess fluorescence at the ocular surface is to use a blue exciter filter over the white light source and a complementary yellow or orange barrier filter over the slit-lamp objective. This highlights the fluorescence and makes staining more easily visible, particularly over the bulbar conjunctival surface. In the absence of a yellow barrier filter, the scatter of blue light from the sclera greatly reduces the contrast of fluorescein staining.

Many slit-lamps have built in “cobalt” blue exciter filters. Determination of the transmission characteristics of this filter will allow the selection of an appropriate complementary yellow barrier filter to observe fluorescence, but usually, a standard yellow barrier filter will suffice (Table 2). Ensuring that the transmission wavelengths of the exciter and barrier filters overlap as little as possible reduces pseudofluorescence, enhances contrast, and improves detection of staining.¹²

The absorption peak of fluorescein sodium occurs between 465 and 490 nm, and the emission peak between 520 and 530 nm.^{10,13} A suggested filter pair for detection of fluorescein staining is a yellow, Kodak Wratten 12 barrier filter (transmitting above 495 nm) or an orange Wratten 15 filter (transmitting above 510 nm) in combination with a blue Wratten 47 or 47A exciter filter. The 47A filter was designed especially to stimulate fluorescein fluorescence and shows greater transmittance than the Wratten 47 over the absorption range.

Where more light is required for photographic purposes, narrow-bandpass interference filters can be used.¹⁴ Another approach is to use a white light source and a blue-green filter over the objective lens (peak transmission at 508 nm), which transmits wavelengths that the dye absorbs. With this technique, conjunctival staining appears black against a green background.¹⁵

The use of both exciter and barrier filters allows both the cornea and conjunctiva to be assessed using a single stain. This is a major advantage in clinical trials where it is otherwise customary to employ fluorescein to grade corneal staining and rose Bengal or lissamine green to grade conjunctival staining.

Disadvantages of Fluorescein Staining

The staining must be read immediately after the FBUT measurement so that the areas of staining are sharply delineated. Once diffusion has occurred, the original pattern of staining may be obscured.

The fluorescent tear film itself may obscure the staining pattern because, over a range of concentrations around 0.1%, fluorescence is proportional to the thickness of the tear film. Therefore, the thicker the tear film at the time of observation, the more the stain pattern will be obscured by tear film fluorescence. If the patient is asked to blink a few times, this permits tear film fluorescence to be distinguished from its staining.

ROSE BENGAL

Rose Bengal is a fluorinated dye. When supplied in drop form it stings greatly on instillation and induces reflex tearing. In the past this was attributed to pH, but current commercial preparations are formulated at close to physiologic pH. Rose Bengal is, however, intrinsically toxic.^{16,17} In fact, it has been shown to suppress human corneal epithelial cell viability *in vitro*; lissamine green does not have this effect and stains the same

areas stained by rose Bengal, which has led many to recommend the clinical use of lissamine green over rose Bengal in evaluation of ocular surface disorders.¹⁸ The staining that rose Bengal dye produces on the conjunctiva and cornea is dose dependent.^{19,20} If drop size (or drop concentration) is reduced to minimize stinging on instillation, the amount of staining is also reduced. The best staining results are achieved by instilling a full drop (eg, 25 μ L) of a proprietary preparation into the conjunctival sac. Because rose Bengal stings, its instillation is best preceded by a topical anesthetic.

Instillation Technique

A drop of sterile, mild anesthetic is instilled into the conjunctival sac, and the patient is allowed to recover from the mild degree of stinging. Recovery is very rapid. (If desired, after Proxymetacaine, a drop of Amethocaine 1.0% can be instilled, which provides a deeper level of anesthesia and further protects against marked stinging from the rose Bengal.)

A drop of rose Bengal 1.0% is instilled onto the surface of the upper bulbar conjunctiva with the upper lid retracted and the patient looking downward.

Note that because both drop and anesthetic may stimulate reflex tearing, the test should follow measurement of the FBUT and measurement of the Schirmer test. (Conjunctival staining from insertion of the Schirmer paper can usually be distinguished from that related to the dry eye disease.) A suggested order of dry eye assessment procedures is included later in this document.

Preventing Overspill

If the rose Bengal drop spills over onto the lid skin, which is common, this will produce a cosmetically unacceptable staining of the skin that may last for several hours. The following method can minimize such overspill:

1. Before instilling the rose Bengal drop, pull the lower lid down to make more space in the lower fornix of the conjunctival sac.

2. With the eye looking down, retract the upper lid and instill the drop onto the upper bulbar surface, letting it flush down over the conjunctival and corneal surface. The rose Bengal collects in the lower fornix.
3. Let the patient blink gently. In this way the upper lid draws rose Bengal from the lower fornical pool over the surface of the globe and distributes it over the whole of the ocular surface.
4. Before releasing the lower lid, place a tissue at the temporal lid margin to mop up excess rose Bengal.
5. Let the patient close the lid and release the retracted lower lid slowly, while simultaneously drawing up the excess rose Bengal into the tissue paper by capillary action.
6. When you think that you have removed all the excess rose Bengal, let the patient blink freely but then mop up the further excess of dilute rose Bengal that appears with a tissue.

This should minimize skin staining and is quicker to do than it sounds.

Both eyes may be stained before grading because there is no risk of the staining pattern in the first eye being obscured by the time the second eye is graded.

Visibility

Rose Bengal staining on the conjunctiva shows up well against the light scattered from the white sclera. It may also show up well on the cornea against a light blue iris background. However, with a dark brown iris background the staining pattern is difficult to see. A red-free (green) light source may enhance contrast of rose Bengal staining over the sclera. Alternatively, a white light source may be used, with a green filter over the objective lens transmitting over the range 545–567 nm, which is absorbed by rose Bengal. Conjunctival staining appears black against a green background.¹⁵ A suitable filter is a Kodak Wratten 58.

Phototoxicity

Rose Bengal is intrinsically toxic, but it can also be photoactivated by ultraviolet radiation, which can cause further toxicity.¹⁷ Photoactivation by sunlight will increase symptoms postinstillation, especially in dry eye patients with heavy staining. Patients in this situation may report prolonged eye pain following rose Bengal instillation. This postinstillation pain can be minimized by liberal irrigation with normal saline at the end of the test.²¹

LISSAMINE GREEN

Lissamine green is a synthetic organic acid dye with two aminophenyl moieties.²² It stains the eye in a similar manner to rose Bengal. However, it is less toxic and consequently less irritating to the ocular surface.²³ In most patients, lissamine green 1.0% will be tolerated as well as fluorescein sodium. As with rose Bengal, lissamine green staining is dose-dependent. Therefore, if a tiny volume is instilled into the conjunctival sac, the staining that occurs will be very limited.

Lissamine green is readily available in impregnated strips or may be ordered as a preprepared 1.0% solution from some pharmacists. Whether the solution or the wetted strips are used, because the dye does not sting, there is no need to limit dose volume, and a full drop should be instilled (say 25 μ L) to maximize the staining observed.

Instillation Technique

The instillation procedure for lissamine green solution is identical to that used for rose Bengal, and the same precautions to limit skin staining should be applied. It should be noted that impregnated strips are also available.

Visibility

As with rose Bengal, lissamine green staining is easily visible on the conjunctiva. On the cornea, staining is seen well against a light blue iris background but is poorly vis-

ible against a dark brown iris background. For both rose Bengal and lissamine green, because the dyes are poorly seen within the tear film, the dye in the tear film does not obscure the staining pattern. Also, because both dyes do not diffuse into the substantia propria of the conjunctiva, the staining pattern is retained longer. Using a white light source and a red filter over the objective lens, transmitting over the wavelengths absorbed by lissamine green (634–567 nm), conjunctival staining appears black against a red background.¹⁵ A suitable filter is a Hoya 25A or a Kodak Wratten 92.

SELECTION AND SEQUENCE OF TESTS FOR DRY EYE ASSESSMENT

The order of tests used in dry eye diagnosis is critically important because each test may influence the next. In general it is good practice to work from the least invasive to the most invasive test. The following clinical tests are presented in order of increasing invasiveness. This is not a comprehensive list but simply a representative sample of tests frequently performed. Because some of the tests are mutually exclusive, it is not suggested that all tests in each section should be performed.

Symptoms and History

A record is required of clinical history and ocular symptoms. A number of authors have developed validated questionnaires for the assessment of dry eye.^{24–29} None of these questionnaires attempts to distinguish symptoms arising specifically from the lids (as might occur in blepharitis) from those arising solely from the ocular surface.

Noninvasive Tests

A number of tests are regarded as noninvasive although, because they involve patient positioning and examination with a light source, it must be accepted that there may be a minimally invasive element. It is likely, too, that timing of the tests may deter-

mine whether they are conducted in steady-state conditions. Standardization of the environment and conditions of measurement are most likely to reduce variation caused by such factors.

The noninvasive tear breakup test (NIBUT) was created to measure the stability of the precorneal tear film.³⁰ It involves projection of a target onto the convex mirror surface of the tear film and recording the time taken for the image to break up after a blink. The test was originally performed with a custom-built “Toposope” but has also been performed over a limited zone of the exposed precorneal film, using the keratometer.³¹ It can be conveniently measured using the TearscopePlus™, which is commercially available.³²

Tear film lipid layer interferometry examines and grades tear film lipid thickness on the basis of the observed interference colors and patterns. Currently available apparatus includes the TearscopePlus™ and the Kowa DR-1 (specular reflection video recording system).³³

Reflective meniscometry is used to measure the curvature of the lower tear meniscus, from which meniscus volume can be approximately calculated and total tear sac volume inferred.^{34,35}

Minimally Invasive Tests

The following tests may be performed immediately after the performance of noninvasive tests. Certain tests involve the instillation of fluorescein. As noted earlier, the amount instilled can be controlled using a micropipette.

1. Fluorescein tear breakup time is a measure of tear film stability.^{36,37} The timing of breakup of the precorneal tear film after the blink is recorded.
2. Grading staining after fluorescein instillation has already been discussed. As noted, the FBUT and staining patterns should be recorded in sequence, first on one eye and then on the fellow eye, recording the FBUT first.

3. Meniscus profile can be photographed after fluorescein instillation and used to estimate meniscus volume and related parameters following a suitable interval after fluorescein instillation.³⁸
4. Instillation of fluorescein is required for the measurement of tear turnover using fluorophotometry and other methods of tear fluorescein clearance.³⁹⁻⁴³

Studies of the inferior tear meniscus have indicated that, in normal eyes, without punctal occlusion, tear meniscus height is restored to normal within 5 minutes after the instillation of a 5- μ L drop of normal saline.⁴⁴

On the basis of this, a 5-minute gap is recommended before the next tests.

Mildly Invasive Tests

Meibometry is a quantitative method for the measurement of the basal, or casual, meibomian lipid levels on the lid margin. It involves application of a plastic loop to the middle third of the slightly everted lower lid. The change in optical density produced by the lipid imprint is used as an index of the casual lipid level.⁴⁵ Modification of this technique to measure lipid delivery involves baseline removal of lipid using a lipid solvent.⁴⁶ This will induce reflex tearing and is moderately to markedly invasive.

A 5-minute gap is recommended before the next set of tests.

Moderately Invasive Tests

A number of tests involve the insertion of a wick into the lower conjunctival sac and measurement of wetting length over a defined period of time.

The phenol red thread test (PRTT) is acknowledged to be less invasive than the Schirmer test. The wetting of a cotton thread was originally regarded as an index of tear volume,^{47,48} probably influenced by the balance between negative hydrostatic pressure in the meniscus and the capillary action of the thread.^{49,50}

Markedly Invasive Tests

The Schirmer I test without anesthetic uses standard filter papers to assess reflex tear production.^{51,52} Insertion of the filter paper causes surface epithelial damage at the site of insertion, which will stain with dyes (eg, rose Bengal). This damage also results in the leakage of plasma proteins into the tear fluid.⁵³

The Schirmer I test of basal tear secretion with anesthetic resembles the test without anesthesia except that it involves the instillation of an unspecified volume of topical anesthetic before the test, which is mopped out of the conjunctival sac before the test.⁵⁴ The efficiency of the latter maneuver in removing fluid is not known, and no validation of the Schirmer test with anesthetic appears to have been reported.

The Schirmer II test measures the tear response to nasal stimulation.^{55,56}

Additional Invasive Tests

Lissamine green and rose Bengal dyes, using either drops or impregnated strips, may be regarded as moderately to markedly invasive tests because they induce reflex tearing. Instillation of rose Bengal, as discussed earlier, must be regarded as a highly invasive test, which, in the absence of anesthesia, induces pain and marked reflex tearing.

Lid Assessment and Meibometry

Clinical assessment of the lid margins, oil glands, and tarsal conjunctiva involves significant lid manipulation, which must be assumed to induce reflex tearing. This applies to a lesser extent to the performance of meibography.⁵⁷ Therefore, such tests must be performed at the end of an examination sequence. A similar argument applies to the assessment of tear oil quality, which involves expression of the oil by pressure through the lids.⁵⁸

Other Ocular Assessments

For clinical trial purposes it is usual to include a full assessment of the anterior seg-

GRADING OF CORNEAL AND CONJUNCTIVAL STAINING
OXFORD SCHEME




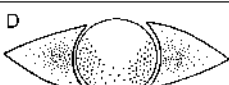
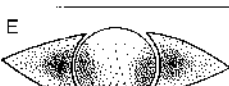
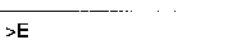
PANEL	GRADE	VERBAL DESCRIPTOR
	0	Absent
	I	Minimal
	II	Mild
	III	Moderate
	IV	Marked
	V	Severe

FIGURE 4. It is suggested that this enlarged, simplified version of the grading scale page be scanned and reproduced for clinical use.

ment (including eversion of the upper lids), measurement of the intraocular pressure, and an assessment of the media and fundus oculi, with or without mydriasis. These tests would normally be performed at the end of the "dry eye" sequence or at a separate visit.

CONCLUSION

Correct use of ocular surface stains in conjunction with appropriate lighting and filters can greatly enhance the detection of staining. The use of the Oxford Grading Scale (Fig. 4) may be copied for the reader's use) offers one approach to the assessment of ocular surface damage. This is useful not only in the initial diagnosis of dry eye but also in the monitoring of ocular surface disease over time. The use of vital stains in conjunction with a battery of tests will facilitate the diagnosis and monitoring of dry eye.

REFERENCES

- Pflüger. Zur Ernährung der cornea. *Klin Monatsbl Augenheilkd.* 1882;20:69-81.
- Joyce P. Corneal vital staining. *Ir J Med Sci.* 1967; 500:357-367.
- Hardman-Lea S, Mengher L, Davey C, et al. Rose Bengal and fluorescein staining in the assessment of keratoconjunctivitis sicca (abstract). *Ophthalmol Res.* 1987;19:19-20.
- Bron A. Reflections on the tears. A discourse on the classification, diagnosis and management of dry eye. The Doynce Lecture. *Eye.* 1997;11:583-602.
- CCLRU. *CCLRU Grading Scales.* Sydney, Australia: Cornea and Contact Lens Research Unit, School of Optometry University of NSW (available through Vistakon), 1996.
- Chong T, Simpson T, Fonn D. The repeatability of discrete and continuous anterior segment grading scales. *Optom Vis Sci.* 2000;77:244-251.
- Efron N, Morgan B, Katsara S. Validation of grading scales for contact lens complications. *Ophthalm Physiol Opt.* 2001;21:17-29.
- Eliason J, Maurice D. Staining of the conjunctiva and conjunctival tear film. *Br J Ophthalmol.* 1990;74: 519-522.
- Norn M. Congo red vital staining of cornea and conjunctiva. *Acta Ophthalmol (Copenh)* 1976;1976:4.
- Maurice D. The use of fluorescein in ophthalmological research. *Invest Ophthalmol.* 1967;6:464-477.
- Thomas M, Szeto V, Cheslyn M, et al. Sequential staining: the effects of sodium fluorescein, osmolarity, and pH on human corneal epithelium. *Optom Vis Sci.* 1997;74:207-210.
- Wolfe D. Fluorescein angiography basic science and engineering. *Ophthalmology.* 1986;93:1617-1620.
- Jacobs J. Fluorescein sodium—what is it? *J Ophthalmic Photogr.* 1992;14:62.
- Erol O, Spira M. An improved method for fluorescein dye photography. *Plast Reconstr Surg.* 1981;68: 120-121.
- Song A, Schmidlap B, Bradley A, et al. Filters for enhanced viewing of conjunctival staining with rose Bengal, lissamine green and fluorescein. *Optom Vis Sci.* 1999;76(suppl):231.
- Toda I, Tsubota K. Practical double vital staining for ocular surface evaluation. *Cornea.* 1993;12:366-367.
- Feenstra R, Tseng M. What is actually stained by rose Bengal? *Arch Ophthalmol.* 1992;110:984-993.
- Kim J, Foulks G. Evaluation of the effect of lissamine green and rose Bengal on human corneal epithelial cells. *Cornea.* 1999;18:328-332.
- Norn M. Rose Bengal vital staining. Staining of cornea and conjunctiva by 10 percent rose Bengal, compared with 1 percent. *Acta Ophthalmol (Copenh).* 1970;48:546-559.
- Snyder C, Paugh J. Rose Bengal dye concentration and volume delivered via dye-impregnated paper strips. *Optom Vis Sci.* 1998;75:339-341.
- Whitcher J. Clinical diagnosis of the dry eye. *Int Ophthalmol Clin.* 1987;27:7-24.
- Norn M. Lissamine green. Vital staining of cornea and conjunctiva. *Acta Ophthalmol (Copenh).* 1973; 51:483-491.
- Manning F, Wehrly S, Foulks G. Patient tolerance and ocular surface staining characteristics of lissamine green versus rose Bengal. *Ophthalmol.* 1995; 102:1953-1957.

24. McMonnies C. Key questions in a dry eye history. *J Am Optom Assoc.* 1986;57:512-517.
25. McMonnies C, Ho A. Patient history in screening for dry eye conditions. *J Am Optom Assoc.* 1987;58:296-301.
26. Schiffman R, Christianson M, Jacobsen G, et al. Reliability and validity of the ocular surface disease index. *Arch Ophthalmol.* 2000;118:615-621.
27. Vitali C, Bombardieri S, Moutsopoulos H, et al. Preliminary criteria for the classification of Sjogren's syndrome. Results of a prospective concerted action supported by the European Community. *Arthritis Rheum.* 1993;36:340-347.
28. Schein OD, Muñoz B, Tielsch JM, et al. Prevalence of dry eye among the elderly. *Am J Ophthalmol.* 1997;124:723-728.
29. McCarty C, Bansal A, Livingston P, et al. The epidemiology of dry eye in Melbourne, Australia. *Ophthalmology.* 1998;105:1114-1119.
30. Mengher L, Bron A, Tonge S, et al. A non-invasive instrument for clinical assessment of the pre-corneal tear film stability. *Curr Eye Res.* 1985;4:1-7.
31. Hirji N, Patel S, Callander M. Human tear film pre-rupture phase time (TP-RPT)—a non-invasive technique for evaluating the pre-corneal tear film using a novel keratometer mire. *Ophthalmic Physiol Opt.* 1989;9:139-142.
32. Guillon J. Use of the Tearscope Plus and attachments in the routine examination of the marginal dry eye contact lens patient. *Adv Exp Med Biol.* 1998;438:859-867.
33. Yokoi N, Takehisa Y, Kinoshita S. Correlation of tear lipid layer interference patterns with the diagnosis and severity of dry eye. *Am J Ophthalmol.* 1996;122:818-824.
34. Yokoi N, Bron A, Tiffany J, et al. Reflective meniscometry: a non-invasive method to measure tear meniscus curvature. *Br J Ophthalmol.* 1999;83:92-97.
35. Yokoi N, Bron A, Tiffany J, et al. Reflective meniscometry a new field of dry eye assessment. *Cornea.* 2000;19(suppl 1):s37-s43.
36. Norn M. Outflow of tears and its influence on tear secretion and break up time (B.U.T.). *Acta Ophthalmol (Copenh).* 1977;55:674-682.
37. Lemp M, Goldberg M, Roddy M. The effect of tear substitutes on tear film break-up time. *Invest Ophthalmol.* 1975;14:255-258.
38. Mainstone J, Bruce A, Golding T. Tear meniscus measurement in the diagnosis of dry eye. *Curr Eye Res.* 1996;15:653-661.
39. Afonso A, Monroy D, Stern M, et al. Correlation of tear fluorescein clearance and Schirmer test scores with ocular irritation symptoms. *Ophthalmology.* 1999;106:803-810.
40. Jones D, Webber W, Smith A, et al. Ophthalmic fluorophotometry: a new solid state fluorophotometer. *J Biomed Eng.* 1982;4:113-117.
41. Pearce E, Keenan B, McRort C. An improved fluorophotometric method for tear turnover assessment. *Optom Vis Sci.* 2001;78:30-36.
42. Macri A, Pflugfelder S. Correlation of the Schirmer 1 and fluorescein clearance tests with the severity of corneal epithelial and eyelid disease. *Arch Ophthalmol.* 2000;118:1632-1638.
43. Macri A, Rolando M, Pflugfelder S. A standardized visual scale for evaluation of tear fluorescein clearance. *Ophthalmology.* 2000;107:1338-1343.
44. Oguz H, Yokoi N, Kinoshita S. The height and radius of the tear meniscus and methods for examining these parameters. *Cornea.* 2000;19:497-500.
45. Chew C, Hykin P, Jansweijer C, et al. The casual level of meibomian lipids in humans. *Curr Eye Res.* 1993;12:255-259.
46. Chew C, Jansweijer C, Tiffany J, et al. An instrument for quantifying meibomian lipid on the lid margin: the Meibometer. *Curr Eye Res.* 1993;12:247-254.
47. Kurihashi K, Yanagihara N, Honda Y. A modified Schirmer test: the fine-thread method for measuring lacrimation. *J Pediatr Ophthalmol.* 1977;14:390-397.
48. Hamano H, Hori M, Hamano T, et al. A new method for measuring tears. *CLAO J.* 1983;9:281-289.
49. Yokoi N, Kinoshita S, Bron A, et al. Tear meniscus changes during cotton thread and Schirmer testing. *Invest Ophthalmol Vis Sci.* 2000;41:3748-3753.
50. Tomlinson A, Blades K, Pearce E. What does the phenol red thread test actually measure? *Optom Vis Sci.* 2001;78:142-146.
51. Schirmer O. Studien zur Physiologie und Pathologie der Tränenabsonderung und Tränenabfuhr. *Albrecht Von Graefes Arch Klin Exp Ophthalmol.* 1903;56:197-291.
52. van Bijsterveld O. Diagnostic tests in the sicca syndrome. *Arch Ophthalmol.* 1969;82:10-14.
53. Stuchell R, Feldman J, Farris R, et al. The effect of collection technique on tear composition. *Invest Ophthalmol Vis Sci.* 1984;25:374-377.
54. Clinch T, Benedetto D, Felberg N, et al. Schirmer's test. A closer look. *Arch Ophthalmol.* 1983;101:1383-1386.
55. Pavan-Langston D. *Manual of Ocular Diagnosis and Therapy*, 4th ed. Boston: Little, Brown and Co, 1996:15-16.
56. Tsubota K. The importance of the Schirmer test with nasal stimulation. *Am J Ophthalmol.* 1991;111:106-108.
57. Mathers W, Shields W, Sachdev M, et al. Meibomian gland dysfunction in chronic blepharitis. *Cornea.* 1991;10:277-285.
58. Shine W, McCulley J. Meibomian gland triglyceride fatty acid differences in chronic blepharitis patients. *Cornea.* 1996;15:340-346.