ORIGINAL ARTICLE

Timothy J. Bennett, CRA, FOPS

Penn State Milton S. Hershey Medical Center
Department of Ophthalmology
P.O. Box 850
Hershey, PA 17033
717/531-5516
717/531-5009 Fax
tjbennett@psu.edu

Gary E. Miller, CRA

Geisinger Medical Center
Medical Photography 01-05
North Academy Avenue
Danville, PA 17822
717/271-6450
570/271-7146 Fax
gmiller@geisinger.edu

Lissamine Green Dye – An Alternative to Rose Bengal in Photo Slit-Lamp Biomicrography

Abstract

Vital stains are often employed in the evaluation and documentation of ocular surface disorders. Rose bengal has traditionally been used to stain devitalized areas of the cornea and conjunctiva as well as precipitated mucous on the corneal surface. Lissamine green has been suggested as both an alternative and an adjunct to rose bengal as a vital stain. Blue-green in color, lissamine green exhibits a staining pattern similar to rose bengal, but is less irritating to patients and does not exhibit anti-viral activity.

In order to discover how ophthalmic photographers could best utilize these stains, a photographic comparison of rose bengal and lissamine green was conducted using Zeiss photo slit-lamps and Kodak Ektachrome Professional films.

Results of this subjective comparison suggest that each dye has advantages in specific photographic situations. Since the staining patterns of rose bengal and lissamine green are essentially identical, it is recommended that these dyes be used interchangeably, based on visual contrast with the prevalent subject color.

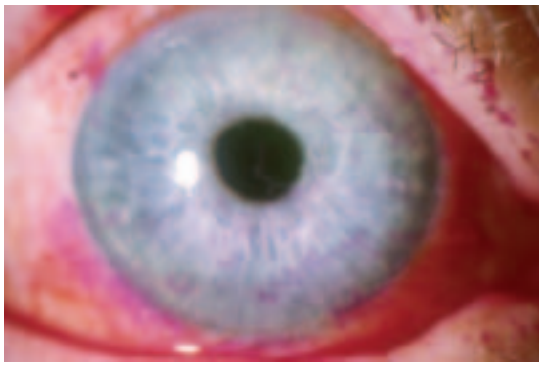

Figure 1: Herpes simplex dendrites stained with rose bengal.

Figure 2: Lissamine green dye used to stain an eye with keratoconjunctivitis sicca. Note the "blue" color of the dye stained tear film.

Rose Bengal

The topical application of vital stains can be a useful diagnostic aid in the evaluation and documentation of ocular surface disorders. Rose bengal is a vital stain that has long been used for this purpose.[1,2] A synthetic derivative of fluorescein that appears red-magenta in color, rose bengal stains devitalized areas of the cornea and conjunctiva as well as precipitated mucous.[3] It is particularly useful in diagnosing keratoconjunctivitis sicca,[4,5] HSV keratitis,[6] (Figure 1) and squamous cell metaplasia.[7] The conventional understanding of the staining mechanism of this dye has changed over the years, indicating that it is not truly a vital stain. For practical purposes however, it performs like one. Rose bengal has been shown to stain normal, healthy cells in vitro, but does not stain normal cells in clinical use, probably due to the protective nature of the pre-ocular tear film.[8] Despite its usefulness in examination of ocular surface disease, there are some disadvantages to the use of rose bengal as a vital stain. Rose bengal is known to cause patient discomfort, and it possesses antiviral properties that could interfere with the results of viral cultures obtained after instillation of this dye.[9]

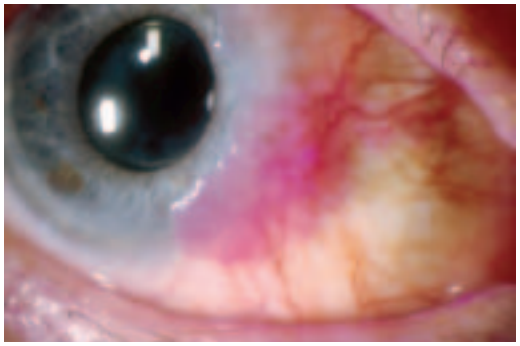

Figure 3a: Devitalized areas of a squamous cell lesion stained with rose bengal. The visibility of the dye is partially obscured by the similar red color of the injected conjunctival vessels.

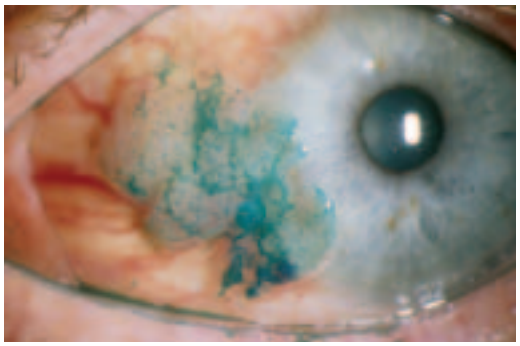

Figure 3b: A similar squamous cell neoplasm stained with lissamine green demonstrating good visual contrast between dye and the lesion.

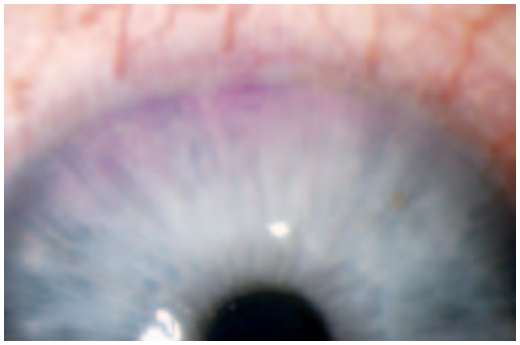

Figure 4a: High magnification view of rose bengal punctate staining in superior limbal keratoconjunctivitis (SLK).

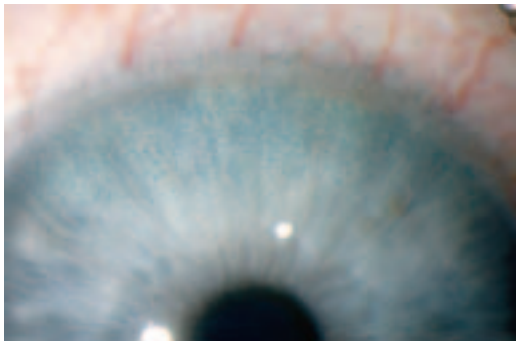

Figure 4b: Same eye stained with lissamine green.

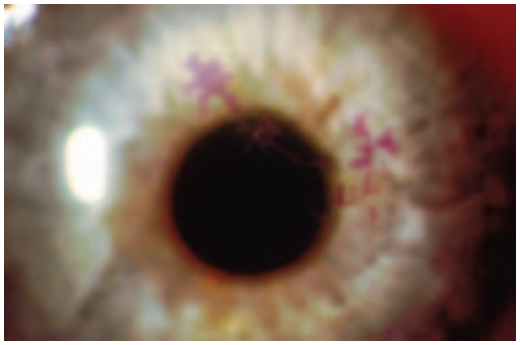

Figure 5a: HSV dendrites stained with rose bengal.

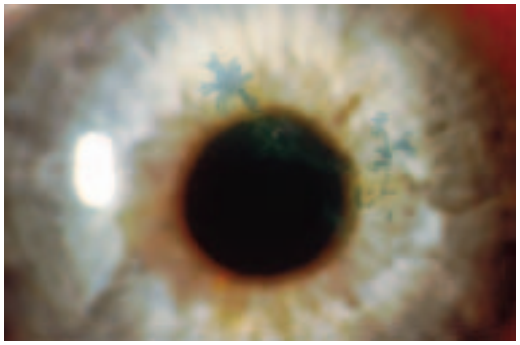

Figure 5b: Same eye stained with lissamine green. Lissamine green stain appears less prominent against the background of the light colored iris.

Lissamine Green

In 1973, Norn first suggested the use of lissamine green as both an alternative and an adjunct to rose bengal as a vital stain.[10] Lissamine green is a synthetically produced organic dye approved by the FDA for use as a colorant in food, drugs and cosmetics. In clinical use, it exhibits staining characteristics similar to rose bengal. Although it's name suggests a green appearance, lissamine green actually appears more blue than green when used to stain the ocular surface (Figure 2). In certain situations, this color may be preferable to that of rose bengal. The blue-green color exhibits a greater visual contrast in red, inflamed eyes. Lissamine green has been shown to be non-irritating to patients in comparison to rose bengal.[11] Despite its reported advantages, lissamine green has yet to gain general acceptance in North America[11] and is little known within the ophthalmic photography community. For a brief period of time, lissamine green was available in the United States as a colored eye lubricant formula in a concentration of .5% from Dakryon Pharmaceuticals. It is now available in sterile strips impregnated with 1.5 mg of lissamine green and is currently distributed it in the United States by Contact Lensmart, Inc.

We have investigated the use of lissamine green as an alternative to rose bengal in photographic applications (Figure 3). Accurate photodocumentation of the staining patterns of these two dyes is dependent on a number of factors. Film choice, illumination technique, proper exposure level and the amount of dye delivered to the eye all effect the photographic results. Based on our brief experience with lissamine green as an alternative to rose bengal, we present the following initial impressions and recommendations.

Methods

At the request of our respective cornea services, we have photographed approximately 40 eyes stained with lissamine green. These patients presented with various ocular surface disorders that traditionally would have been stained with rose bengal for photodocumentation. In some cases we made direct comparisons between lissamine green and rose bengal by alternating both dyes on the same eye, irrigating between applications (Figures 4 & 5).

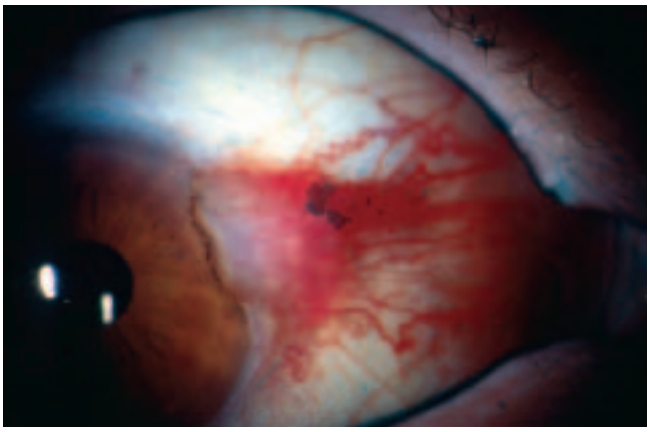

Figure 6: Underexposed photograph of lissamine green stained squamous cell lesion. Note the dull green appearance of the dye due to underexposure.

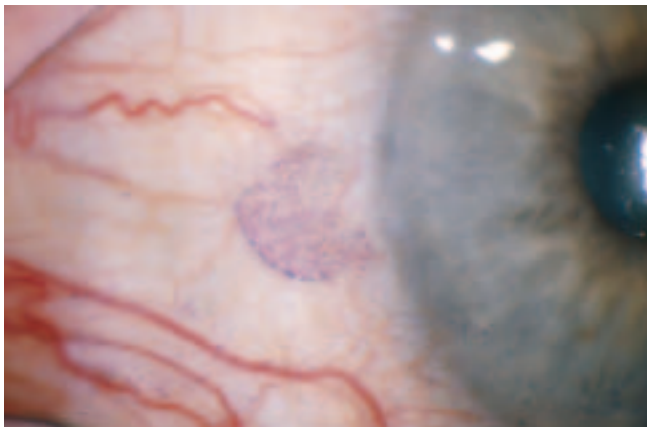

Figure 7: Conjunctival intraepithelial neoplasm (CIN) stained with a combination of both rose bengal and lissamine green to create a deep blue stain color.

All photographs in this comparison were taken with Zeiss photo slit-lamps using Kodak Ektachrome daylight balance color slide films (Ektachrome 100 Professional and E200 Professional). We purposely avoided overly saturated emulsions that are popular for general pictorial use. These emulsions often exhibit a warm balance and can exaggerate certain colors. For accurate and consistent color reproduction, Kodak recommends the use of Ektachrome Professional films with a neutral balance and normal saturation in most scientific and medical applications.[12] The differences in exposure and saturation we present in comparing these two dyes could vary with other films.

When instilling a vital stain prior to photography, it is easier to control the amount of dye delivered to the ocular surface by using sterile strips. In liquid form, it is easy to deliver too much dye. Faulkner and Varley[13] suggest that "less is more" when using vital stains. This is especially true in photographic situations. The conjunctival sac cannot hold the amount of dye typically delivered in a full drop. Subtle findings can easily be masked unless all surplus dye is irrigated before viewing. Unnecessary staining of the external lid surfaces can also occur as a result of excess dye and irrigation.[14] Periorbital staining does not interfere with clinical observation, but is aesthetically undesirable in a photograph.

The sterile strip should be moistened with a drop of sterile irrigating or saline solution. To instill the dye, retract the lower lid and gently touch the palpebral conjunctiva with the pre-moistened strip. The patient is then instructed to blink a few times to distribute the dye across the corneal surface. Some practitioners will moisten the strip with a drop of topical anesthetic in place of saline when using the more irritating rose bengal.*

We have found that both of these dyes are best photographed using diffuse or broad beam, focal illumination. In most situations, diffuse illumination is the best choice, especially when rendering subtle staining patterns. The contrast provided by strong directional lighting is usually unnecessary and can distract from the color contrast provided by the stain. Proper exposure is essential in capturing the sometimes-subtle staining patterns of these vital stains. Diffuse illumination provides greater exposure latitude with color transparency films. Rose bengal seems to tolerate and sometimes even benefit from a slight amount of underexposure.[15] In our experience, the same does not hold true for lissamine green, especially with E200 film. Underexposure causes lissamine green to appear more like its' concentrated form: a dark, dull green instead of the usual saturated blue color (Figure 6). An exposure increase of up to one half f-stop may be necessary for eyes stained with lissamine green. We recommend bracketing exposures to establish a standard exposure range that works well for both dyes.

Discussion

It is important to consider the differences between clinical observation and photographic documentation when employing these dyes. The three-dimensional dynamic view through the binocular biomicroscope often provides enough visual depth to separate a staining pattern from the background of underlying surfaces. In a static two-dimensional photograph, the background color can easily mask the staining characteristics of a particular dye when they are similar in color. Therefore, color choice becomes a more important consideration when applying vital stains specifically for photography.

Since the staining patterns of rose bengal and lissamine green are essentially identical, the choice of stain is simply one of color preference. Lissamine green provides another color in the palette of stains available to the ophthalmic photographer. We suggest choosing the dye that will provide the best visual contrast for a given photographic situation. We agree with Norn's assertion that lissamine green is a better choice when viewing or photographing a red, inflamed eye.[10] The similarity in color between rose bengal and inflamed tissues in a red eye does not provide enough color contrast between stained

*As with all diagnostic and therapeutic agents, these ophthalmic preparations should only be administered under the orders and direction of a physician.

Figure 8: External photograph of the lissamine green stained interpalpebral exposure zone in a patient with keratoconjunctivitis sicca.

areas and the dominant background color. Conversely, rose bengal is the better choice when trying to image subtle corneal defects in an eye with a blue iris as the background. In cases with a brown iris as the background, both dyes work well, with lissamine green offering just a slight advantage. Norn also suggested the use of both dyes, combined to create a deep blue or purple color (Figure 7). We found no real advantage to this combination, but it does offer the ophthalmic photographer another choice if rose bengal or lissamine green are too similar in color to the subject to provide adequate color contrast.

These are subjective impressions, but photo slit-lamp biomicrography is as much an art as science. Obtaining successful photographs is often dependent on the experience and subjective intuition of the photographer.

Conclusion

When ocular surface disorders are documented photographically, rose bengal is often the vital stain of choice. However in some circumstances, the magenta color of rose bengal does not provide adequate color contrast for proper visualization. Lissamine green, a lesser-known vital stain with its blue-green appearance and nearly identical staining pattern, can be an effective substitute for rose bengal in these situations (Figures 8 & 9). The key element is to determine which stain will provide the best color contrast for each photographic situation.

Table 1: Suggested Dye Selection for Slit-Lamp Photography

Subject	Rose Bengal	Lissamine Green
Inflamed red eye		✔
Keratoconjunctivitis sicca		✔
Precipitated mucous	✔	
Corneal staining (herpetic dendrite or punctate staining) in eye with blue iris	✔	
Corneal staining (herpetic dendrite or punctate staining) in eye with brown iris	✔	✔
Conjunctival lesion (squamous cell or CIN)		✔

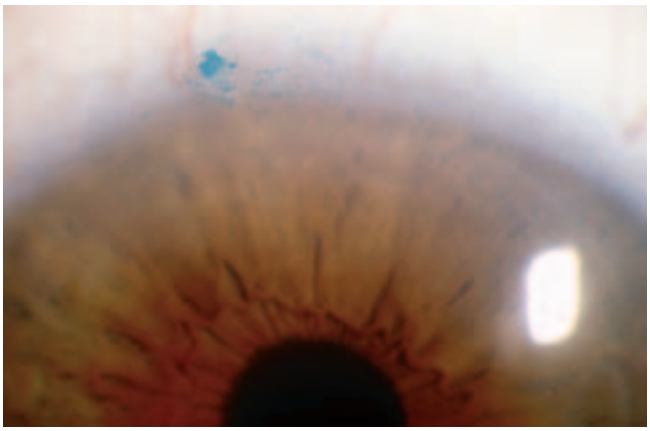

Figure 9: Prominent lissamine green staining of the limbal area in superior limbal keratoconjunctivitis. Lissamine green provides excellent color contrast between the stained area and the adjacent looped vessels characteristic of SLK.

References

1. Sjögren H. Zur Kenntnis der Keratoconjunctivitis Sicca(Keratitis filiformis bei Hypofunktion der Tränendrüsen). *Acta Ophthalmol.* 1933;suppl
2. Passmore JW, King JH Jr. Vital staining of conjunctiva and cornea: review of literature and critical study of certain dyes. *Arch Ophthalmol* 1955;153:568574.
3. Norn MS. Vital staining of the cornea and conjunctiva. *Acta Ophthalmol.* 1962;40:389-401.
4. O'Day K. Bengal rose as an aid in the diagnosis of 'kerato-conjunctivitis sicca"(Sjögren's syndrome). *Med J Aust.* 1951;2:708-709
5. Lannsche RK. Vital staining in normal eyes and in keratoconjunctivitis sicca. *Am J Ophthalmol.* 1965;60:520-525.
6. Wilson FM II. Rose bengal staining of epibulbar squamous neoplasms. *Ophthalmic Surg.* 1976;7:21-23
7. Marsh RJ, Fraunfelter FT, McGill JI. Herpetic corneal epithelial disease. *Arch Ophthalmol.* 1976;94:1899-1902.
8. Feenstra RPG, Tseng SCG. What is actually stained by rose bengal? *Arch Ophthalmol.* 1992;110:984-993.
9. Roat MI, Romanowski E, Auraullo-Cruz T, Gordon J. The antiviral effects of rose bengal and flourescein. *Arch Ophthalmol.* 1987;105:1415-1417.
10. Norn MS. Lissamine green. Vital staining of the cornea and conjunctiva. *Acta Ophthalmol.* 1973;51:483-491.
11. Manning FJ, Wehrly SR, Foulks, GN. Patient tolerance and ocular surface staining characteristics of lissamine green versus rose bengal. *Ophthalmology.* 1995;102:1953-1957
12. Eastman Kodak Company. *KODAK Scientific Imaging Products.* Publication L-10,1989, pg 47.
13. Faulkner WJ, Varley GA. Corneal diagnostic techniques. In: Krachner JH, Mannis MJ, Holland EJ. eds. *Cornea: Volume I, Fundamentals of Cornea and External Disease.* St. Louis: Mosby-Year Book, Inc., 1997: 275-281.
14. Wiffen S, Barry CJ. Rose bengal staining of the ocular surface. *J Ophthalmic Photography.* 2001;23:2;73-75
15. Mártonyi CL. Photography of the cornea and external eye. In: Krachner JH, Mannis MJ, Holland EJ. eds. *Cornea: Volume I, Fundamentals of Cornea and External Disease.* St. Louis: Mosby-Year Book, Inc., 1997: 283-304.