ORIGINAL RESEARCH

Red-free versus cobalt blue illumination in fluorescein diagnostic staining of the external ocular surface

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Abstract

Aim: To investigate the usefulness of the red-free filter of the Haag-Streit slit lamp 900 in fluorescein diagnostic staining of the external ocular surface.
Patients and methods: One author examined 200 consecutive patients (371 eyes) requiring diagnostic staining of the external surface with fluorescein dye. The evaluation was carried out using the Haag-Streit slit lamp 900 BM. Both red-free and cobalt blue filters were used in succession after the saline-moistened fluorescein strip was applied to the conjuctival sac. The initial filter used for each patient was randomly determined. The appearances of the tear film and of the areas of dye uptake were noted, as was the ease with which the associated lesions and the ocular structures were visualized.
Results: The red-free filter was found to be superior to the cobalt blue filter in all clinical situations except for the assessment of tear film break-up time. The superiority of the red-free illumination in fluorescein diagnostic staining of the external ocular surface is due to its ability to produce intense fluorescence without obscuring the details of the associated anterior ocular structures.
Conclusion: The use of the red-free filter in place of the traditionally used cobalt blue filter greatly enhances the diagnostic capability of fluorescein staining of the external ocular surface and deserves to be used more widely.

Key words: *Fluorescein, Ocular surface*

Introduction

Fluorescence is the phenomenon of absorbing certain wavelengths of light and then emitting a light of a longer wavelength. The use of fluorescein in diagnostic procedures for ocular surface disorders is widespread.

Traditionally slit lamp examination of the fluorescein stained ocular surface is carried out using cobalt blue illumination. The red-free filter of the Haag-Streit slit lamp provides a green light that is also capable of exciting intense fluorescence.[1] The relatively longer wavelength of the red-free illumination allows added visibility of the anterior ocular structures. Red-free light applanation tonometry has been shown to achieve optimal visualization of the tear menisci and therefore more accurate estimation of the intraocular pressure.[1] However, in external surface evaluations requiring the use of fluorescein, the role of the red-free filter has remained unexplored. The purpose of this study is to report on the usefulness of the red-free filter in comparison to the cobalt-blue filter in clinical situations.

The relevant information was gained using binocular slit lamp biomicroscopy, which takes place in 3 dimensions and

allows dynamic evaluation of the surface. This is in contrast to slit lamp photography, which is a 2-dimensional documentation with a small depth of field and many different variables of illumination and contrast.

Patients and methods

Between October 1999 and May 2000, one observer examined 200 consecutive patients (371 eyes) undergoing slit lamp evaluation requiring the use of fluorescein. The Haag-Streit slit lamp model 900 BM was utilized for the study. A fluorescein paper strip moistened with saline was applied to the inferior tarsal conjunctiva or to the specific area of interest as in the Seidel's test. For the purpose of illumination, both red-free and cobalt blue filters were used in rapid succession. The choice of the initial filter was determined in a randomized fashion. The slit lamp voltage of 6.0 V was used for all examinations. The angle between illumination and observation was set at 60°. A beam width of 3 to 4 mm. was used to scan the ocular surface. The observations were made through eyepieces devoid of any barrier filter. For the observation under the initial filter, a baseline value of zero was given for each of the following parameters

- generalized fluorescence of the tear film
- focal changes in the fluorescence (areas of dye uptake, negative staining, dye pooling, and dye dilution)
- quality of the contrast for visualization of these focal changes
- visibility of other structures and lesions at surface level and deeper.

On assessment under the subsequent illumination, the arbitrary value of +1, 0, or -1 was given if the parameter assessed was favourably affected, not affected, or adversely affected, respectively, by the change of the filter. Based on these assessments, the filter that gave the optimal performance for each diagnostic test was recorded.

Results

The observations are summarized in **Table 1**. The diagnostic information provided by the red-free filter was superior to the cobalt blue filter in 77% and equally good in a further 14% of all fluorescein dye tests carried out in this study, while the cobalt blue filter was preferred only in 9%.

The nature of the fluorescence

The precorneal tear film, which appeared greenish-yellow under the cobalt blue light, showed bright yellow fluorescence under the red-free light. Areas of corneal epithelial defect, which appeared intensely bright yellow under the red-free light, showed up as relatively less bright and more greenish under the cobalt blue light. Conjunctival defects appeared strikingly bright yellow under the red-free light in contrast to their dull yellow fluorescence under the cobalt blue light. The darker contrasting background of the blue light improved the overall visibility of the corneal and conjunctival dye uptakes. However, due to more intense and bright yellow fluorescence, these areas stood out prominently despite the lesser contrasting background of the red-free illumination.

Surface correlation of the lesions

Both cobalt blue and red-free illumination highlighted areas of fluorescein uptake (for example, punctate epithelial erosions). However, the red-free light additionally permitted visibility of other surface lesions that had not taken up the stain (spots of punctate epithelial keratitis). The use of the red-free filter thus allowed an opportunity for the simultaneous appraisal and integrated overview of both stained and unstained surface abnormalities under a single illumination. A topographical correlation of the stained epithelial defect to surrounding unstained abnormality such as an infiltrate could therefore be conducted with ease under the red-free but not under the cobalt blue illumination.

Corneal surface elevations appeared as featureless dark spots within the fluorescein-stained tear film under the cobalt blue illumination (negative fluorescein staining). The red-free illumination, on the other hand, permitted their actual visibility in 3 dimensions.

Depth correlation of the lesions

Under red-free illumination, it was possible to concomitantly visualize any unstained abnormalities such as infiltrates or abscesses lying beneath the stained surface defect. This was not feasible with the cobalt blue-filtered light due to its poor

Table 1. Slit lamp filters preferred for fluorescein surface diagnostic tests.

Observed condition	No. of eyes	RF > CB [No. (%)]	RF = CB [No. (%)]	CB > RF [No. (%)]
Conjunctivitis and episcleritis	48	48 (100)	0 (0)	0 (0)
Precorneal tear film	80	53 (66)	14 (17)	13 (16)
Tear film break-up	60	16 (27)	24 (40)	20 (33)
Dry eyes	40	40 (100)	0 (0)	0 (0)
Dual staining	21	21 (100)	0 (0)	0 (0)
Corneal elevations	23	23 (100)	0 (0)	0 (0)
Seidel's test	33	20 (60)	13 (39)	0 (0)
Keratitis	50	50 (100)	0 (0)	0 (0)
Chemical burns	16	16 (100)	0 (0)	0 (0)
Total	**371**	**287 (77)**	**51 (14)**	33 (9)

Abbreviations: RF = red-free light; CB = cobalt blue light; > = superiority; = = equality

ocular penetration. Moreover, only the red-free light allowed adequate optical sectioning of the cornea. This helped in clarifying the intrastructural relationships and localization of deeper abnormalities in relation to the surface defects. Thus fluorescein-stained epithelial erosions were appreciated along with deeper lying sub-epithelial infiltrates, and epithelial defects could be seen in accurate relationship to a deeper lying corneal abscess or endothelial plaque.

Correlation with vascularization

The red-free light enhanced visibility of the blood vessels. This enabled the observation of stained surface defects in simultaneous correlation to any vascular structures. The red-free light was therefore advantageous in the study of the conditions where the surface alteration in the dye uptake was accompanied by a vascular change (episcleritis, phlyctenular nodules, keratitis, and so on). Further, red-free light was especially useful in monitoring the progress of corneal ulcers, by facilitating the documentation of the area as well as highlighting any attendant vascularization.

For assessment of chemical burns of the ocular surface, the use of red-free light offered the unique advantage of highlighting any limbal ischaemia, as well as the areas of dye uptake. This facilitated the gradation of the severity of the chemical burns.

Measurement of stained lesions

On the Haag-Streit slit lamp, the vertical control of the slit beam is disabled when the cobalt blue filter is interposed in the illumination system. The control remains operational during the use of the red-free filter, however. Thus, under a single illumination, both accurate measurement as well as optimal visualization of the stained surface defect is ensured if the red-free filter was used.

Dual staining of surface lesions

To study the epithelial changes of herpes simplex keratitis and keratoconjuctivitis sicca, dual staining was carried out using both rose bengal and fluorescein dyes. The cobalt blue illumination only highlighted the areas of fluorescein uptake. It was therefore difficult to distinguish the dark looking areas of rose bengal uptake from the similar looking areas of negative fluorescein staining. The clarification necessitated an additional manoeuvre of switching to the white light. The red-free light, on the other hand, showed up deep purple-looking areas of the rose bengal uptake as well as the bright yellow looking areas of the fluorescein staining.

Seidel's test

Both cobalt blue and red-free light provided satisfactory visibility of the leaking rivulet. Whereas more contrast was obtained with the former, more intense fluorescence was observed with the latter. Due to better visibility of the structures in the background, the red-free light additionally permitted an accurate anatomic correlation of the origin of the leak to the bleb area. It also provided an unexpected vital opportunity to concomitantly appreciate the degree of translucency and the vascularity of the bleb.

Tear film break-up

Due to their darker appearance, areas of tear film break up were easy to visualize under the cobalt blue light. Their identification under the red-free light was facilitated to a large extent by the brighter fluorescence of the surrounding tear film. On occasions (**Table 1**), the underlying ocular structures remaining visible did interfere with their recognition.

Tear film debris

The subtle mucus debris in the fluorescein-stained tear film of dry eyes was remarkably easy to observe under the red-free light due to its intensely bright yellow fluorescence.

Conjunctival papillae and follicles

Pooling of fluorescein in the crevices around these conjuctival elevations was easy to observe with the aid of either filter. Red-free light, however, additionally highlighted the associated pattern of conjunctival vascularization, making the differentiation of the papillae from the follicles easier.

Teaching aid

The use of red-free light on the video-slit lamp was found to be advantageous as a teaching aid. The brighter fluorescence of the surface defects, coupled with the better visibility of the ocular structures in the background made the demonstration of clinical abnormality more effective.

Discussion

The use of fluorescein to evaluate the external ocular surface remains an integral part of clinical practice. The information gathered, however, can be enhanced by the proper understanding of the physicochemical properties of fluorescein and the knowledge of the optical properties of the light filters involved. It is useful to remember that, unlike the filters used in the fundus camera, the slit lamp filters are broadband filters (**Figure 1**). Moreover, slit lamp

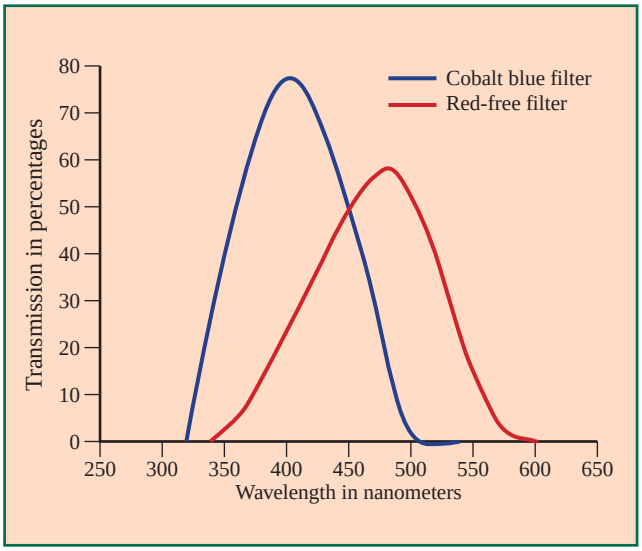

Figure 1. The transmission spectra of colored filters on the Haag-Streit 900BM slit lamp. Reproduced with permission from Haag-Streit AG.

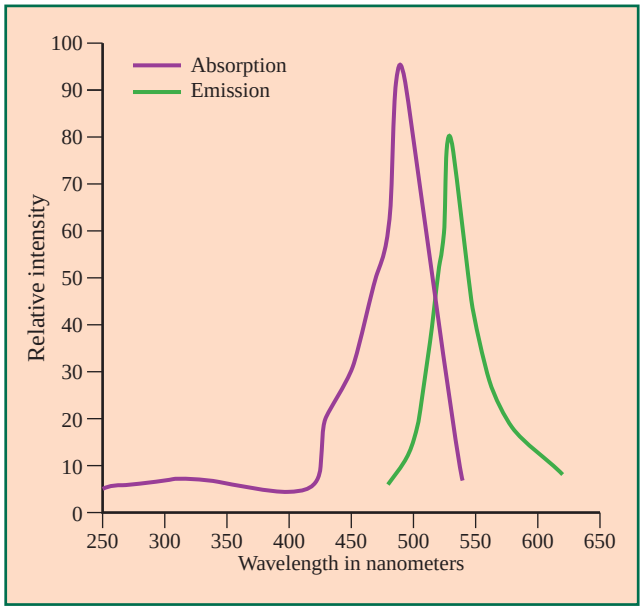

Figure 2. The absorption and emission spectrum of sodium fluorescein. Reproduced with permission from Pearson, 1984.

evaluation of the fluorescein diagnostic staining of the ocular surface almost always takes place without the use of a contrast enhancing barrier filter in the observation eyepieces.

The absorption spectrum of fluorescein in dilute aqueous solution in the pH range of 7.0 to 8.0, is usually shown as a curve with small peaks in the ultraviolet range and a large maximum peak at 490 nm, dropping steeply towards a longer wavelength and ceasing at 530 nm (**Figure 2**).[2] The light of wavelength of 490 nm, which is the peak of the excitation spectrum, produces maximum intensity of fluorescence. The emission curve of fluorescent light has a maximum intensity of emission at 530 nm with a range from 500 to 600 nm (**Figure 2**). The precorneal tear film pH is in the range of 7.4 to 7.5.[3] The fluorescein diagnostic test on the external ocular surface involves dilution of the topically applied fluorescein in the tear film with further dilution occurring due to the tear flow. It is reasonable to assume that the above-quoted absorption and emission spectra are applicable in fluorescein staining of the external ocular surface. The cobalt blue filter on the Haag-Streit slit lamp 900 BM has a peak emission at 400 nm. The red-free filter, on the other hand, has peak emission at 475 nm. (Personal communication, Rene Hecht, 1999.) Since the peak transmission wavelength of the red-free filter is closer to the peak absorption value of fluorescein, the red-free light would produce more intense fluorescence in comparison to the cobalt blue light. The observations described here substantiate these arguments. It is interesting to note that the older Haag-Streit models were fitted with the red-free filter, which had a peak transmission at exactly 490 nm.

The spectral range of fluorescence is dependent on the wavelength of the exciting light.[3] When excited by a light of wavelength of 490 nm, maximum fluorescence occurs in the region of 530 nm. When illuminated by the shorter wavelengths of the cobalt blue-filtered light, the emitted fluorescence is more greenish and less yellowish in appearance. The relatively longer wavelengths of the red-free light produces a bright yellow appearance due to the higher proportion of the correspondingly longer wavelengths in the emitted light.

The ease of viewing of areas of dye uptake is a function of the intensity of the fluorescence in that area and the degree of contrast available in the background. The cobalt blue filter provides better contrasting background whereas the red-free light produces more intense fluorescence. The cobalt blue-filtered light masks the details of the unstained ocular structures. There is therefore a need to switch back and forth between the blue and white light for the proper topographical orientation of the stained lesions to the clinical abnormality as a whole. The longer wavelengths of the red-free light permits view of the structures of the anterior segment due to the better ocular penetration. Since the red-free filtered light produces brighter fluorescence of the areas of the dye uptake and at the same time permits view of the structures in the background, the stained lesions are seen in a proper anatomical as well as clinical context.

The measurement of the tear film break up time involves recognition of the areas of breaks in the fluorescein stained precorneal tear film. The spots devoid of fluorescein within the brightly fluorescent tear film were easy to recognize under the red-free light. However, the advantage gained from the more intense fluorescence of the surrounding tear film was, in part, neutralized by the visibility of the anterior segment structures in the background, which somehow seemed to interfere with the recognition of the areas of the break.

The Seidel's test involves application of a high concentration of fluorescein to the area of suspected fistula. This was achieved by applying the fluorescein paper strip directly to the area under investigation. The identification of the leaking rivulet depends on the dilution by the leaking aqueous. The red-free illumination offered a number of advantages in this situation. The intense yellow fluorescence at the edges of the clear stream made the leaks easily noticeable. Moreover, the localization of the leak to an exact anatomical point on the bleb was feasible, a correlation not easy in the darker background of the cobalt blue illumination. The red-free illumination could be further exploited by obtaining other clinically vital information such as the vascularity and the translucency of the bleb.

The clinical condition of herpes simplex keratitis often necessitates dual staining with rose bengal and fluorescein dyes. The appreciation of the rose bengal-stained areas requires the use of either white or red-free light. The blue light, which is traditionally used to view the fluorescein-stained component, is inadequate for the appreciation of the rose bengal-stained areas. The use of red-free light offers the optimal appreciation of the areas stained with both dyes under a single illumination.

Fluorescein has a role in evaluation of conjunctival papillae. The recognition of low profile papillae is facilitated by fluorescein dye, which outlines the papillae as it lies in the valleys at their bases. The geographic extent of the papillary response and the size of the papillae as well as the presence or absence of the epithelial erosions on the apices of the papillae are important diagnostic features, guiding therapy. Compared to the cobalt blue-filtered light, red-free light provides better visibility of the papillae, as well as the fluorescein pooling around them. For a novice ophthalmologist, the differentiation of the papillae from the follicles can be made easier by the enhanced viewing of the vessels, which occupy the top of the papillae but surround the base of the follicles.

To summarise, the superiority of the red-free illumination in fluorescein diagnostic staining is based on its ability to produce intense fluorescence without obscuring the details of the anterior ocular structures. In addition, red-free illumination is able to highlight any accompanying vascular component. The evaluation of the surface lesions under the red-free light offers the advantages of the brilliant yellow fluorescence of stained areas and the convenience of the evaluation of the abnormality as a whole under a single illumination. The demonstration of fluorescein-stained clinical abnormalities on the video monitor is clearer for students if red-free light is used instead of the cobalt blue-filtered light. For evaluation of ocular surface abnormalities requiring the diagnostic use of staining agents, routine use of the red-free light can be an enlightening clinical experience for even the most experienced ophthalmologhist. The red-free illumination on the Haag-Streit slit lamp provides a wealth of information that otherwise remains obscured during cobalt blue filter illumination.

In conclusion, while white light remains the basis of slit lamp examination, the use of the red-free filter in place of the traditionally used cobalt blue filter enhances the diagnostic capability of fluorescein staining of the external ocular surface, and deserves to be more widely used.

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