Lissamine green where have we been and where are we now?

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Lissamine green – where have we been and where are we now?

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Abstract

The General Optical Council has recently clarified the legal position for eye care practitioners wishing to use lissamine green dye for anterior eye examination. This paper reviews the history and current use of the dye in ophthalmological examination. Though data are limited, the dye is found to be well tolerated by patients when used in strip form. Recommendations are made on the optimum use of the dye based on published research and its unique staining properties are discussed.

Introduction

The use of dyes in ophthalmology dates back to the 1800s when a method of ‘vital staining’ was used to stain cells and tissues in their living state. The three vital ocular dyes that are used in examination of the anterior eye are sodium fluorescein, lissamine green (LG) and rose Bengal. Lately, the use of LG in optical practice has been recommended by researchers and key opinion leaders alike, particularly in the investigation of dry eye. However, until March 2020 the legality of eye care practitioners using the dye in the UK was unclear. This paper seeks to review the historical and current uses of LG, trace the changes to its legal position in the UK, examine the safety profile of the dye, consider how best to optimise its use in optical practice and discuss the advantages of using LG in addition to sodium fluorescein in anterior-eye assessment.

Origin and history of lissamine green

LG, chemical formula C<sub>27</sub>H<sub>25</sub>N<sub>2</sub>NaO<sub>7</sub>S<sub>2</sub>, is a synthetically produced, non-fluorescent, organic dye, a diphenyl-naphthyl methan derivative of the phenylmethane dye and also known as ‘wool green S’ or ‘fast light green’. It has been approved by the US Food and Drug Administration as a colour additive in food and cosmetics. The first recorded use of LG as a staining agent was for proteins in the process of electrophoresis in 1957. In 1967, LG was initially recognised for its ocular use in the determination of endothelial cell viability. However, it was Nom, in 1973, who described its use as a vital dye for staining of the cornea and conjunctiva, highlighting that it stains degenerate cells, dead cells and mucus, with staining properties very similar to those of rose Bengal.

In subsequent years, LG was suggested for the clinical diagnosis of xerophthalmia, though it was shown to lack the required sensitivity, and for the assessment of epithelial damage in cataract surgery. Despite these occasional applications, LG was not widely utilised until the mid-1990s when Tseng confirmed Nom’s findings and affirmed that LG would not stain healthy cells, but was ideal for the detection of dead or degenerate cells.

The recommendation of the use of LG in the Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) I report in 2007 helped establish LG as an important dye in the repertoire of testing regimes used to evaluate dry-eye disease, and this has been further reinforced with publication of the TFOS DEWS II report. Additionally, the Optician magazine published a CET-accredited article on LG in 2010, which suggested the dye was becoming more widespread in clinical practice in the UK.

The legal position in the UK

At a time when LG was gaining greater acceptance as a clinically useful dye, uncertainties were raised over the legality of its use in the UK. To fully understand this legal situation, the problems which arose over the legality of fluorescein strips must be examined initially.

A series of EU directives from 1994 (which are now in the process of being reviewed) have previously defined the role and purpose of what constitutes a ‘medicine’ in comparison to a ‘medical device’. Under these directives a medicine or medicinal product is ‘A substance ... that is intended to treat, prevent or diagnose a disease’. Within the UK, fluorescein is licensed as a Pharmacy (P) medicine, meaning it can be legally used by an optometrist or contact lens optician during the course of professional practice.

In 2013, Bausch & Lomb withdrew its fluorescein-impregnated strips, known as Fluorets, from the market; at the time, these were the only impregnated strips licensed as a P medicine for use in the UK.

As a result, practitioners had no option but to use 1% or 2% fluorescein Minims for clinical practice – the only appropriately licensed products in the UK. The use of Minims has several problems, including difficulties in controlling the amount of fluorescein instilled into the eye. Moistened fluorescein strips are often more appropriate for clinical use in optometric or contact lens-related practice where only a small amount of dye is required. If the concentration of fluorescein is too high, it can delay assessment by 2–4 minutes, until the concentration has reduced to a useful level. The use of 2% Minims or saturated strips (saline not shaken off) can result in these excessive concentrations. Given that a 1% Minim or moistened strip provided a useful level of fluorescence quickly, the cost-effectiveness of fluorescein strips makes them a good choice in clinical practice. However, as the only available CE-marked fluorescein strips were classified as ‘medical devices’ in Europe, there was considerable ambiguity over whether practitioners in the UK could legally use the strips as an alternative. Medical devices are defined as ‘products or equipment intended generally for a medical use’. In response to lobbying from the profession, the Medicines and Healthcare Products Regulatory Agency (MHRA) has agreed not to prevent the supply of CE-marked medical device fluorescein strips to the UK market until its status has been agreed by the EU.

Subsequently, and following expert legal advice and consultation with an expert clinical consensus panel, the General Optical Council (GOC) issued a statement on 30 September 2013 clarifying the
circumstances in which optometrists and contact lens opticians can use CE-marked fluorescein ophthalmic strips, to the effect that.17

- registrants are responsible for acting in their patients’ best interests, in line with the GOC Code of Conduct; and
- acting in patients’ best interests, as required by the Code of Conduct, may make it necessary for registrants to use CE-marked strips (the marketing and supply of which are not currently opposed by the MHRA), where they are acting within their scope of practice.

The guiding principle quoted by the GOC was that optometrists and contact lens opticians should act in the best interests of patients and in doing so it was therefore appropriate to use CE-marked fluorescein strips.17

Clarification around the use of LG was not included in the 2013 GOC statement because LG, unlike fluorescein, did not have P medicine status but was licensed as a medical device in the UK. This resulted in a situation in the UK where (from 2013 to 2020) some practitioners chose to use LG, effectively ‘off-label’, whereas others were reticent to use it at all.

In 2016, the UK College of Optometrists issued a statement, highlighting that it did not advocate the use of LG, citing the GOC statement on fluorescein.18 Without a GOC and MHRA statement the College of Optometrists could not ensure clinicians would not face regulatory action. The College then continued to lobby for a regulatory statement along with the Association of Optometrists and other industry stakeholders.

Following requests from UK optical professional bodies, the GOC formed a panel of academics/researchers and experienced eye care practitioners to help clarify the legal position of LG. The panel met in April 2018 and they unanimously agreed that:

based on the evidence available and practice and clinical opinion, lissamine green is clinically safe to use and that optometrists and contact lens opticians in the UK may, within their scope of practice, use a CE marked lissamine green impregnated ophthalmic strip for clinical investigations of the ocular surface until further notice.19

Despite this, regrettably it was to be almost 2 years before the GOC finally published its position statement on LG use in March 2020.19 In light of the clarification by the GOC, the College of Optometrists’ Director of Policy and Strategy has stated: ‘Our members will welcome the news that they can use CE-marked lissamine green ophthalmic strips in appropriate clinical circumstances.’20

The timeline from 2013 to the present day is summarised in Figure 1.

As with fluorescein, LG strips have been CE-marked in EU member states as medical devices. While the MHRA maintains the strips should be regulated as a medicinal product, they have indicated that they will refrain from taking any regulatory action against the use of such strips. Having considered the panel’s report and the MHRA’s stance the GOC position states:

[We] consider that there will be circumstances where it is necessary, in the patient’s best interests, for optometrists and contact lens opticians to use CE-marked lissamine green ophthalmic strips ... within the scope of their practice.19

An explanation of the patient’s best interests may be found in the GOC statement, which makes the following recommendation:

Registrants are individually responsible for acting at all times in the best interests of their patients, and must determine the most appropriate clinical care in accordance with the GOC’s Standards of Practice for Optometrists and Dispensing Opticians.19

This position was further supported by the Association of Optometrists and their clinical director:

This pragmatic approach will mean that optometrists and contact lens opticians, who deem lissamine green the most appropriate way to assess their patient’s eye health, can do so without being concerned that they may face regulatory action.21

In summary, to understand the legal position regarding the use of ophthalmic dyes in the UK, it should be recognised that, whilst it is possible to purchase, and use, fluorescein and LG-impregnated strips, these do not currently have a UK medicinal licence. However, the GOC and MHRA statement gives the reassurance that practitioners can use fluorescein and LG without the prospect of facing regulatory action.

**Reporting of adverse events with lissamine green**

The ocular surface’s tolerance to LG strips is high but can be lower in individuals with dry-eye disease.22,23 Lower tolerance has also been reported when using higher concentrations of LG solution.24,25

While a handful of studies have reported no significant adverse effects from the use of LG,8,26,27 including two studies which used a 1% LG concentration,8,26 many others have simply not included these data.28-35

Several studies have specifically investigated the tolerance of LG on the ocular surface. Manning et al.23 compared

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**Figure 1.** Timeline from 2013 to present day, summarising the legal position of the use of fluorescein and lissamine green CE-marked strips.
Similarly, Khurana et al.22 used LG in their discomfort was significantly shortened surface assessment. This paper also compromising the quality of the ocular rose Bengal in both groups, without significantly more comfortable than patients. However, LG was found to be stinging with LG than non-dry-eye rose Bengal. Dry-eye patients reported slightly more discomfort were experienced with the 2% group of non-dry-eye subjects. This is compared to irritation in 92% and 49% of subjects in the dry-eye and control groups respectively with rose Bengal.

Korb et al.24 explored patient experience following a combination of different types of dye in different concentrations in eyes affected by ocular surface disease. The results indicated that 1% LG resulted in no adverse sensation but burning and discomfort were experienced with the 2% or 3% concentrations.

A recent Australian study by Delaveris et al.25 investigated the performance of four different brands of LG strips and calculated the concentration of LG created from each strip when dipped into 200 µL of sterile saline for 1 minute. The concentrations ranged from 0.5% to 4.9%. Staining intensity was greater with higher concentrations of LG (brands: Green Glo, Hub Pharmaceuticals, USA and OP Green, Surgtech Innovation, India, 4.9% and 3.4% respectively). The pH of the stained saline was also measured for each of the four brands and the results ranged from 6.26 to 6.75. None of the 22 volunteers reported any discomfort and the researchers reported that the range of pH measured would not be expected to cause ocular surface burns or stinging.

It is also important to remember that if any adverse events do occur in the course of optometric practice they can be reported via the MHRA’s yellow card scheme. This can now be completed online by eye care practitioners (https://yellowcard.mhra.gov.uk/). At the time of writing, to the best of the authors’ knowledge, no adverse events associated with LG had been reported to the MHRA through their yellow card system.

Toxicity

Chodosh et al.36 showed no significant cellular toxicity and no effect on cell viability when an assay was conducted that had previously shown toxicity with rose Bengal. In this assay the corneal epithelial cells were exposed to the dye for 5 minutes then washed away with buffer in a method designed to mimic the natural tearing that would occur in humans. Pilot research involving the analysis of corneal cells from five participants following repeated instillations of 0.5% LG (up to six instillations) showed no significant increase in cell death.

Optimum use of lissamine green

LG has been shown to have a similar staining pattern to rose Bengal, but it is much better tolerated by patients.24 Its best efficacy is in the examination of conjunctival staining, while sodium fluorescein remains the most suitable dye for the visualisation of corneal staining.26 The visualisation of ocular changes in highly vascularised areas, such as those found in lid wiper epitheliopathy (LWE), is easier with LG compared to fluorescein because the green dye provides excellent contrast against the red marginal and palpebral conjunctiva,26 as shown in Figure 2.

Figure 2. (a) Marx’s line stained with lissamine green and (b) lid wiper epitheliopathy stained with lissamine green.

Efron et al.39 highlighted that LG is the most commonly used dye throughout the literature for assessing LWE.

Concentrations of at least 1% LG are recommended to optimise visualisation of the staining patterns.40 Variations may be introduced due to the brand of strip and amount of saline used. A study of LG strips found that the concentrations of BioLissamine (Biotech Healthcare Group, India) and Lissaver (Dina-Hitex Spol, Czech Republic) came in at under 1% (being 0.9% and 0.5% respectively).25 Therefore, the TFOS recommend waiting 5 seconds once the strip has been wetted with a full drop of saline to elute the dye and then ensure a full drop is instilled on to the ocular surface from the strip, as opposed to shaking the solution off the strip as you would with fluorescein. This should maximise the viewing of any staining.26

Further discrepancies may arise depending on how long practitioners wait to examine staining patterns following instillation. The ocular surface should be examined between 1 and 4 minutes post-instillation of the dye. If the surface is examined too soon, any staining pattern present may not have had adequate time to develop fully. After about 4 minutes the staining pattern has been found to fade.40 Foulks40 recommends starting with a low illumination and increasing the brightness until the appearance of any staining is optimum. Examining staining patterns under light that is too bright can reduce the contrast of the pattern, making it more difficult to fully evaluate. The use of a red barrier filter in the observation system of the slit lamp has been shown to significantly improve visualisation of staining.26
For the optimum visualisation of LWE two instillations of LG with two separate strips, 1 minute apart, are recommended followed by viewing between 3 and 5 minutes after the second instillation.38 The method for using LG was clearly outlined by the TFOS; see Table 1 for this step-by-step method.2

When assessing the anterior eye there will be occasions when clinicians wish to utilise both fluorescein and LG dyes because of their respective benefits for observation of corneal and conjunctival staining. Within the published literature there appears to be little consensus regarding any definitive protocol for use in such instances. Research does highlight that sequential staining and/or using more than one strip will increase the likelihood of observing ocular surface damage.42 The DEWS II report summed up the use of LG and fluorescein:

while corneal staining is perhaps a later stage feature of DED [dry eye disease], combination staining with fluorescein and lissamine green instilled by moistened and saturated filter paper strip to highlight corneal and conjunctival / eyelid margin tissue damage is recommended as the most appropriate diagnostic technique for evaluating ocular surface damage.2

Korb et al.24 used a mixture of 2% fluorescein with 1% LG and found that the fluorescent characteristics of fluorescein were not altered by the addition of LG. If dyes are instilled in paper form it would be impossible to guarantee that the fluorescein was at twice the concentration of LG. The importance of this ratio of concentrations has not been investigated.

A study by Bron et al.43 examined the characteristics of fluorescein, LG, and rose Bengal in detail. They found that the properties of LG and fluorescein differ in many ways. LG has a higher molecular mass and binds more strongly to a cell nucleus than fluorescein. The ideal concentration of fluorescein for viewing is about 0.1%, which is 10 times less than a 1% concentration of LG (the minimum recommended concentration). The diffusion gradient would mean that LG (at the higher concentration) would be more likely to enter a cell. Conversely, fluorescein’s lower molecular mass increases its likelihood of entering the cell. Once in the cell LG would bind more strongly to the cell nucleus than fluorescein. Although Korb et al.24 have advocated the simultaneous instillation of the two dyes, given their different chemical properties, sequential instillation may be better.

Six studies reported instilling fluorescein prior to the instillation of LG,31,33,35,41,44,45 however none of the methodologies describe the rationale for this order. Guillon and Maissa46 instilled LG before fluorescein, but similarly there is no explanation as to why this order was chosen. In a case report by Maldonado-Codina et al.,47 the staining was examined firstly using fluorescein followed by LG at one visit and then the order of dye instillation was reversed at a subsequent visit (on a separate day). The authors found that the order in which the dyes were instilled did not have any impact on the staining pattern, but it should be noted this case report examined only four eyes of two patients.47 Accordingly, current literature does not appear to provide unambiguous clinical guidance on a recommended order of instillation when utilising both fluorescein and LG to assess potential ocular surface damage to both the cornea and conjunctiva.

Does lissamine green enhance the investigation of ocular surface staining?

Originally, rose Bengal was used to visualise conjunctival staining, but its potential adverse impact on healthy corneal cells48 and poor tolerance by some patients (in particular those with dry eye)35,24 led clinicians to seek better-tolerated alternatives such as LG.6 However, if an eye care practitioner is already using sodium fluorescein dye, is there any benefit to also using LG?

Korb et al.24 compared corneal and conjunctival staining detected by rose Bengal, fluorescein, LG and combinations thereof in a small cohort of 14 participants. The best efficacy was found with a mixture of rose Bengal and fluorescein. However, as rose Bengal is not well tolerated, the authors recommended using a mixture of fluorescein and LG, which they found to be more efficacious than fluorescein alone, particularly with respect to conjunctival staining. More recent supportive evidence for LG use was reported for a larger cohort (n = 50) where LG conjunctival staining was found to be one of the most sensitive metrics for assessing the treatment effects of artificial tears.45

The need for using a combination of dyes was demonstrated in a study designed to examine the relationship between lid margin staining and sensitivity. Researchers used both fluorescein and LG to examine the margins for staining on 27 eyes of 27 subjects.41 The final staining score used for comparison with sensitivity was whichever was the highest (the fluorescein staining score or the LG staining score). The paper does not indicate which dye gave the highest score either on individual subjects or in general, but the use of both dyes was deemed necessary to elicit the full extent of lid margin staining.

### Table 1. Step-by-step guide to the use of lissamine green (LG)

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>For installation, wet the LG strip with saline, with the whole drop retained on the strip for at least 5 seconds to elute the dye.</td>
</tr>
<tr>
<td>2</td>
<td>10 µL, or 1/4–1/5 drop, appears optimal volume if pipetting a specific concentration.</td>
</tr>
<tr>
<td>3</td>
<td>The drop is instilled inside the lower temporal lid on upgaze with the eyelid pulled temporally to avoid damage to the conjunctiva or lid wiper tissue.</td>
</tr>
<tr>
<td>4</td>
<td>Observation should occur between 1 and 4 minutes post-instillation,60 with use of a red filter to potentially aid visualisation29</td>
</tr>
<tr>
<td>5</td>
<td>For lid wiper epitheliopathy, assessment involves repeat instillation of LG using two separate strips, wetting with two saline drops, with viewing recommended after 3–6 minutes67</td>
</tr>
</tbody>
</table>

Lissamine green – where have we been and where are we now?
Additionally, a study investigating conjunctival staining and its association with dry-eye symptoms applied LG followed by fluorescein on the conjunctiva of non-contact lens wearers (72 subjects) and soft contact lens wearers (102 subjects). The authors found that, in the non-contact lens wearers, higher levels of both fluorescein and LG staining were associated with increased dry-eye symptoms. However, in the soft contact lens wearers, only LG staining acted as a discriminant between the symptomatic and non-symptomatic dry-eye patient, indicating its usefulness in this patient group and the benefits of using both dyes routinely in clinical practice.

In 2015, Eom et al. examined conjunctival staining as imaged with LG and the same staining imaged with fluorescein and a yellow barrier filter. The authors concluded that fluorescein with the filter was more effective than LG. However, the sample size was small (13 eyes of 13 subjects), no barrier filter was used to view the LG staining and effectivity was based on the extent of staining shown by each dye. The authors do concede that the two dyes stain slightly different cell types.

In a case report of solution-induced corneal staining (SICS), researchers found that LG staining corresponded closely to the white-light observations of SICS, whereas fluorescein staining was more extensive. This case report only included four eyes of two subjects. It was speculated that the larger area observed with fluorescein might be due to it diffusing laterally within the epithelial tissue, or it may be due to fluorescein binding to preservatives from contact lens care solutions which are dispersed across the epithelial surface. The authors suggested that the use of more than one type of ophthalmic dye may be useful in understanding the underlying mechanism of this phenomenon.

In the first DEWS report in 2007, it was noted that fluorescein diffuses rapidly into tissue and therefore any staining observed with fluorescein must be graded quickly. LG staining, however, persists for longer, which is particularly advantageous for grading and photography. In addition, the TFOS DEWS II pathophysiology report in 2017 also highlights that corneal filaments stain particularly well with LG (Figure 3). There is no mention of fluorescein in relation to the staining of filaments. Furthermore, a recent study examining possibilities of differentially diagnosing Sjögren’s dry eye from non-Sjögren’s found that bulbar conjunctival LG staining was significantly higher in patients with Sjögren’s dry eye. It should be noted that it is still not conclusive as to exactly how LG interacts with living cells and therefore there is still a degree of uncertainty as to what LG staining actually represents.

**Summary**

The GOC has made clear that LG may be used where it is in ‘the patient’s best interests’ and optometrists and contact lens practitioners in the UK can be assured that no regulatory action will be taken for use of an LG strip in ‘appropriate clinical circumstances’. This paper has highlighted studies which show that LG stains differently to fluorescein and several studies in which the authors recommend the use of LG as an adjunct to fluorescein.

In regard to safety, with the exception of one study, in which the concentration and form of LG used were unclear, the evidence suggests prepared solutions of LG can cause minor ocular irritation even at concentrations as low as 1% but significantly less irritation than with rose Bengal. By comparison, there does not appear to be irritation from LG instilled into the eye via strips, even with concentrations as high as 4.9%. A possible reason for this may be that the additives in the dye to keep the strip sterile have the fortunate side effect of improved patient tolerance to the dye. Alternatively, it could be hypothesised that the irritation reported from LG solutions may be related to the solvent used to create the solutions.

Based on the evidence above, and consistent with the GOC statement, the recommendation of this review would be for eye care practitioners who wish to use LG to examine conjunctival staining to continue doing so with LG-impregnated paper strips (LG in solution form is not currently available in the UK, to the best of the authors’ knowledge), using an approved protocol or methodology as described previously. Optometrists and contact lens opticians who have previously been hesitant or reluctant to use it should now feel confident using it with the GOC’s backing and renewed understanding of its applications. Fluorescein would still be required, and is preferable, as a staining agent for the cornea.
Conclusion

Given the growing evidence base on the use of LG, its benefits to clinical practice and tolerability, the clarification from the GOC should serve as reassurance for practitioners wishing to use LG in routine practice, particularly in the investigation of dry eye. The summary from the MHRA as published by the GOC is as follows:

The MHRA accepts that there may be instances where companies may supply lissamine green opthalmic strips that have been CE-marked in EU member states, and has said that at the present time, no regulatory action will be taken against strips that are CE-marked as medical devices.

Providing that the product being used is CE-marked the current lack of licensing in the UK should not prevent optometrists and contact lens opticians from using lissamine green in clinical practice.18

Although there is a general consensus that there are likely to be minimal adverse effects from LG use in strip form, establishing a larger evidence base could help to better inform clinical decisions. Whilst some questions remain unanswered with regard to the availability and the performance of differing strips, evidence indicates that LG plays a valuable role in aiding our evaluation and understanding of ocular surface damage and as such it should be viewed as in the patient’s best interest to utilise such techniques.

Relevance to practice

- This paper explains the advantages of using LG dye in conjunction with fluorescein as part of any anterior-eye assessment
- Recommendations are made to use LG in strip form rather than in solution
- The safety profile of lissamine green is reviewed

Acknowledgements

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References


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CET multiple choice questions

This article has been approved for one non-interactive point under the GOC’s Enhanced CET Scheme. The reference and relevant competencies are stated at the head of the article. To gain your point visit the College’s website college-optometrists.org/oip and complete the multiple choice questions online. The deadline for completion is 31 July 2021. Please note that the answers that you will find online are not presented in the same order as in the questions below, to comply with GOC requirements.

1. According to a study by Delaveris et al., which brand of lissamine green strip produced the highest concentration of dye?
   • Green Glo
   • Biotech
   • OP Green
   • Lissaver

2. Staining patterns visualised using lissamine green should be viewed:
   • Immediately post-instillation of the lissamine green dye
   • Using a red barrier filter in the observation system of the slit lamp
   • Five minutes post-instillation of the lissamine green dye
   • Using a yellow barrier filter in the observation system of the slit lamp

3. Lissamine green has similar staining properties to which other dye?
   • Rose Bengal
   • Sodium fluorescein
   • Tryptan blue
   • Alcian blue

4. The guiding principle of the GOC is that practitioners should:
   • Keep all invasive tests to an absolute minimum
   • Only use non-CE-marked dyes where a CE-marked equivalent is unavailable
   • Follow best international practice when in doubt
   • Act in the best interests of the patient

5. Which of the following is a guideline from TFOS regarding the best technique for using lissamine green?
   • Dip the lissamine green strip into saline for about a minute
   • Place the dye on the superior bulbar conjunctiva to examine tear dynamics
   • Check for staining immediately post-instillation
   • Instil two wetted strips of lissamine green to check for lid wiper epitheliopathy

6. Which of the following is true regarding the examination of solution-induced corneal staining (SICS) according to a 2013 case report?
   • SICS is best examined exclusively with fluorescein
   • SICS is best examined exclusively with rose Bengal
   • SICS is best examined with a combination of stains
   • Lissamine green can cause an apparent increase in staining area in SICS

CPD exercise

After reading this article, can you identify areas in which your knowledge of lissamine green has been enhanced?

How do you feel you can use this knowledge to offer better patient advice?

Are there any areas you still feel you need to study and how might you do this?

Which areas outlined in the article would you benefit from reading in more depth, and why?