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Comparative performance of lissamine green stains

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ABSTRACT

Purpose: To investigate the performance of lissamine green strips from different manufacturers. Additionally, the repeatability, need for sequential dye instillation and impact of repeated lid evertion on lid wiper staining were assessed.

Methods: Study 1 was a prospective, randomised cross-over study where controlled volumes of lissamine green solution prepared from strips (Biotech, Lissaver, GreenGlo, OPGreen) were instilled (right eye: single; left eye: double instillation) on five different days, with OPGreen being tested twice. Lids were everted and digital photographs taken, which were later assessed by a masked observer. Study 2 was an investigator-masked, randomised, controlled study testing the impact of single versus repeated lid evertion. Lid wiper staining was graded (0 to 3 in 0.5 steps).

Results: Lid wiper staining differed significantly between lissamine green solutions, with GreenGlo showing the highest amount of staining, and Lissaver the least (all $p > 0.009$). There were no differences in lid wiper staining over two days, using the OPGreen solution (all $p > 0.05$). The number of drops instilled (single versus double) did not significantly affect lid wiper staining (all $p > 0.05$). Repeated lid evertion increased lid wiper staining ($p = 0.007$ when combined with double drop instillation). Light absorbance patterns and measured concentrations aligned with clinical findings.

Conclusion: There were significant differences in performance between lissamine green solutions. Lid wiper staining was impacted by repeated lid evertion but sequential instillation and use of the Korb grading scale provided little advantage over simpler methods Clinicians must consider this when investigating lid wipers, especially when interpreting a negative finding.

1. Introduction

The “lid wiper” region of the eyelid has become an area of interest in the ocular surface field. There have been reports of higher rates of lid wiper staining or lid wiper epitheliopathy in those with dry eye and in contact lens wearers, but this evidence remains equivocal [1–5]. Large variations exist in the techniques used to examine and stain the lid wiper region [1,6]. Anecdotal observations by the authors have suggested that some strips of lissamine green may not stain as vividly as others. This study’s overarching aim was to assess various techniques for lid wiper staining detection, in order to establish a reliable method for future planned studies [5]. Specific aims included (1) to compare the performance of lissamine green strips from different manufacturers in detecting lid wiper staining; (2) to assess the day to day repeatability of lid wiper staining measurements; (3) to evaluate potential differences between single and double dye instillation; and (4) to evaluate the impact of repeated lid eversion on lid wiper staining.

2. Methods

All procedures were conducted in accordance with the tenets of the Declaration of Helsinki and conformed to the National Health and Medical Research Council Statement on Human Experimentation. Written informed consent was obtained after explanation of all study procedures, approved by the Human Research Ethics Committee at UNSW Sydney (The University of New South Wales).

Participants who required use of eyedrops other than saline or eye ointments during the study period and participants with any ocular pathology were excluded from both studies. The lissamine green strips used for this study had a nominal lissamine green concentration of 1.5 mg per strip. To ensure that a standard volume of lissamine green was instilled into the eyes, test solutions were prepared by dipping each strip for one minute in 200ul of sterile saline (0.9% NaCl, Astra Zeneca, NSW, Australia) as previously described [4,5]. Solutions were prepared by investigators not involved in the assessment and grading of lid wiper

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staining visibility (IJ, US). Fourty microlitres of lissamine green solution were instilled into the lower conjunctival sac of each eye using a calibrated micropipette and sterile tips.

2.1. Study 1: effect of different lissamine green strips

Study 1 aimed to assess the effect of different lissamine green solutions on lid wiper staining and its day to day repeatability. A prospective, randomised, contralateral, double masked study involving five visits on five separate days was conducted. All visits were conducted during daytime hours between 10 o'clock and 3 o'clock to minimise the potential for any diurnal variation of lid wiper staining to affect measurements. Participants of any sex aged 18 years and older were enrolled. Four different lissamine green solutions were tested, including Biotech (Biotech Vision Care Pvt, Ltd, India), Dina Strip Lissaver-Plus (Dina-Hitex spol.s.r.o, Czech Republic), GreenGlo (HUB Pharmaceuticals, LLC, USA), and OPGreen (Ophthetics Unlimited, India). OPGreen was tested on two separate days to assess repeatability.

A drop of lissamine green solution was instilled in each eye and participants were instructed to gently close and rotate their eyes. After one minute, digital photography of the right eye was performed following lid evertion. A second 40ul drop of lissamine green solution was instilled solely into the left eye 5 min following the first instillation. One minute later, digital photography of the left eye was performed. Each photograph received a unique identification code.

Lid wiper staining was graded by a single experienced, masked investigator (AD) after data collection using the uniquely coded digital photographs. Grading was conducted under constant viewing conditions which included a single computer monitor and standard room lighting. Photographs were graded using Korb's grading scale [3], with the length and width grades averaged to form a single score termed "Korb score". On a separate occasion, using the same images but in different order, lid wiper staining was graded using a simplified single pictorial scale where 0 = no staining, 1 = mild staining, 2 = moderate staining, and 3 = severe staining in 0.5 steps, which was termed "simplified score" (Fig. 1). The pictorial scale was designed so that each image or step corresponds to the equivalent average Korb score.

2.2. Study 2: effect of single versus repeated lid evertion

Study 2 aimed to evaluate the effect of repeated lissamine green instillation and repeated lid evertion on lid wiper staining. A prospective, randomised, controlled, investigated-masked study involving two visits was conducted. Ten participants took part in the study.

Table 1
Example flowchart: Effect of single versus repeated lid evertion on lid wiper staining. The order of procedures and eye to be tested were randomised.

Control eye		Test eye	
Visit 1	• 1 drop of lissamine green • Lid evertion and grading - *After 5 min* • 1 drop of lissamine • Lid evertion and grading	Visit 1	• 1 drop of lissamine green • No lid evertion - *After 5 min* • 1 drop of lissamine green • Lid evertion and grading
Visit 2	• 1 drop of lissamine green • Lid evertion and grading - *After 5 min* • 1 drop of lissamine • Lid evertion and grading	Visit 2	• No lissamine green • Lid evertion - *After 5 min* • 1 drop of lissamine • Lid evertion and grading

Table 1 details the flow chart of the procedures tested in this second study. The order of testing and the eye to be tested were randomised. An investigator not involved in the assessment and grading of lid margin staining prepared and instilled a solution of OPGreen lissamine green before calling a second investigator in the room to conduct grading. In addition, two separate investigators graded the first and the second evertion of the double drop, double evertion visits to ensure that the 'end of procedure grading' was done by the same single masked investigator and that this investigator remained masked to the testing procedure being assessed throughout the study period.

To estimate the true concentration of the prepared solutions described above, a standard curve was created by preparing lissamine green solutions in concentrations of 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml, 2 mg/ml, and 125 mg/ml (Lissamine Green B, Sigma-Aldrich, MO, USA). The true concentration of solutions made from the four different lissamine green strips (preparation details given above) was back-calculated based on the standard curve. Light absorption spectra of the lissamine green strips were measured in solutions made with 0.1 M phosphate buffered saline (pH 7.2) with a spectrometer (UV/VIS/NIR; TECAN safire^2) between 280 and 1000 nm. The pH of each lissamine green solution was also measured.

Due to their categorical nature, data were analysed using Wilcoxon signed rank and Friedman tests, after testing for outliers. Outliers were identified through the calculation of z-scores and applying a 99% confidence interval. To assess for individual differences in staining properties between strips, paired *t*-tests were carried out and the significant *p*-value was adjusted to 0.009 to account for the multiple comparisons of the *post-hoc* test. The repeatability of lid wiper staining over two days was assessed using Wilcoxon signed rank test. The

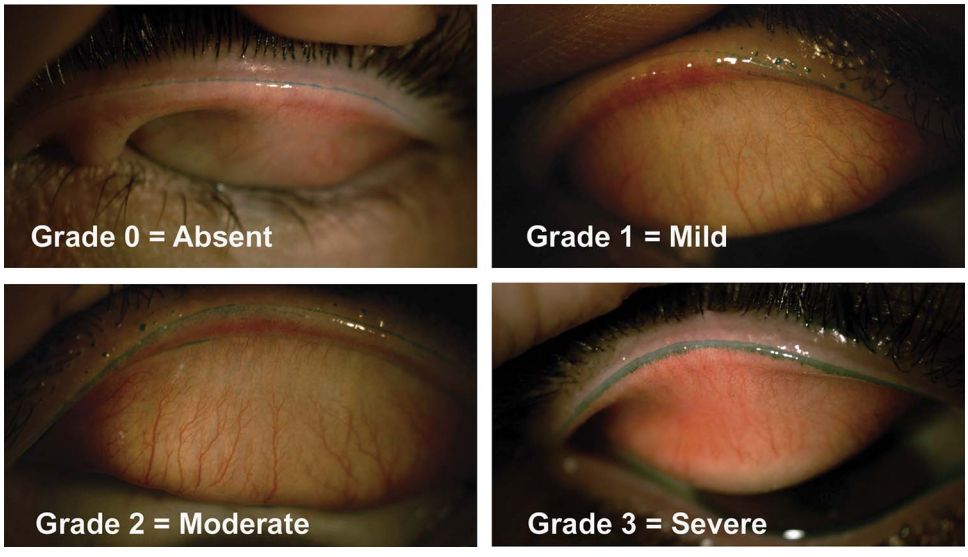

Fig. 1. A 0 to 3 pictorial grading scale of lid wiper staining was used to obtain a "simplified score" where 0 = absent, 1 = mild, 2 = moderate, 3 = severe. Clinicians were instructed to grade in 0.5 steps and to carefully differentiate lid wiper staining from Marx's line staining, which is a feature in most eyes including those without any lid wiper staining (for example, top left panel).