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REVIEW

Lissamine Green in Ophthalmology: A Comprehensive Review of Diagnostic and Clinical Applications

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ABSTRACT

Lissamine green (LG), a diagnostic dye that stains devitalized or damaged epithelial cells, is widely used to assess ocular surface integrity, enabling the detection of inflammation, epithelial defects, and conjunctival irregularities. To explore the diagnostic and clinical applications of LG in ophthalmology, focusing on its use for ocular surface diseases, a group of experts in ophthalmology and optometry participated in an advisory board to share their clinical practice

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experience with the use of LG. Building on the advisory board contents, this narrative review was based on a combination of expert opinions from the roundtable discussion and a comprehensive review of the current literature. This review highlights the clinical relevance of LG as a diagnostic tool in ocular surface disease and underscores the potential of newer formulations to enhance diagnostic accuracy. In particular, the review highlights the historical development of LG use in ophthalmology and its advantages over other dyes, especially in terms of patient comfort and safety, as well as specific clinical protocols for using LG in assessing dry eye disease severity and inflammatory responses. Additionally, the review examines recent advancements in LG formulations, which enhance their utility in clinical practice, and addresses safety considerations. Potential areas for future research are also discussed, particularly in developing standardized evaluation procedures using artificial intelligence.

Keywords: Lissamine green; Ophthalmology; Ocular surface staining; Tear film assessment; Clinical protocols; Contact lenses

Key Summary Points

Lissamine green (LG) is a diagnostic dye used for assessing ocular surface integrity by selectively staining devitalized or damaged epithelial cells, making it an essential tool for diagnosing and monitoring ocular surface diseases, including dry eye disease (DED).

LG staining is widely employed for evaluating inflammation, epithelial defects, and conjunctival irregularities, aiding in the differential diagnosis of conditions such as allergic conjunctivitis, superior limbic keratoconjunctivitis, and contact lens-induced ocular surface changes.

LG is well tolerated, with minimal adverse effects, and has been shown to be less irritating than rose bengal, reinforcing its role as a preferred diagnostic dye for ocular surface evaluation.

A newly developed liquid LG formulation offers improved staining consistency and ease of application, overcoming limitations of traditional LG strips and enhancing diagnostic precision in clinical practice.

INTRODUCTION

Ocular surface diseases encompass a broad spectrum of disorders, ranging from common conditions, such as dry eye disease (DED), to more severe conditions, such as corneal ulcers. Accurate diagnosis of ocular surface diseases is of paramount importance for several reasons. Firstly, misdiagnosis or delayed diagnosis can lead to prolonged discomfort, exacerbation of symptoms, and potentially corneal complications with irreversible vision loss. Secondly, many ocular surface diseases share overlapping clinical presentations, making differential diagnosis challenging. Thirdly, appropriate management strategies hinge upon precise identification of the underlying pathology, emphasizing the need for targeted and personalized treatment approaches.

Despite various methods available to diagnose ocular surface disease in clinical practice [1], cost and time often constrain the number of tests carried out during first-line visits. However, ocular surface staining with fluorescein and lissamine green (LG) represents a simple, inexpensive, rapidly performed, and evidence-based assessment commonly available in most eye clinics [2]. In addition to LG and fluorescein, rose bengal has historically been used to detect devitalized cells and mucin deficiency. Although effective, its higher cytotoxicity and associated discomfort have limited its routine clinical use in favor of better-tolerated alternatives such as LG. Thus, using dyes to assess ocular surface staining is recommended for widespread adoption to provide

a comprehensive evaluation of ocular surface health and improve the diagnostic process.

In particular, Norn proposed the clinical use of LG as a diagnostic dye for the cornea and conjunctiva in 1973 [3]. Research has shown that staining with LG correlates with the presence of inflammatory cells and cytokines [4, 5]. This suggests its potential use as a biomarker for assessing ocular surface inflammation.

To expand knowledge on the use of LG for diagnostic purposes, a group of experts in ophthalmology and optometry participated in an advisory board to share their insights and clinical experience. Building on the advisory board contents, the purpose of this narrative review is to summarize the most recent evidence on this topic, with a particular focus on applications of LG for the diagnosis of ocular surface disease and its use in allergic disease and contact lens (CL) practice. Safety considerations and clinical protocols are also discussed, including an overview of currently available LG formulations.

METHODS

A group of experts in ophthalmology and optometry participated in an advisory board to share their clinical practice experience with the use of LG. Building on the advisory board contents, this narrative review was based on a combination of expert opinions from the roundtable discussion and a comprehensive review of the current literature. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors. All experts involved in the advisory board are authors of the paper.

Narrative Review

Given the narrative nature of this review, a formal systematic review protocol and PRISMA flowchart were not applied. A comprehensive search of the PubMed/MEDLINE database was conducted. The search strategy utilized a combination of keywords related to LG (e.g., "LG staining," "ocular surface staining") and its clinical application (e.g., "LG and ocular surface disease," "LG and inflammation," "LG and allergic conjunctivitis," "LG and contact lens"). Inclusion criteria encompassed peer-reviewed articles published in English from inception to April 2024 that explored the use of LG in ophthalmology. Additionally, reference lists of relevant articles were manually searched to identify additional studies. According to the narrative review format, no formal exclusion criteria were applied beyond language and relevance to the review's objectives. The synthesis of findings involved a narrative approach, in which themes and patterns across studies were identified and discussed in relation to the research question. Quality assessment of included studies was not conducted, given the narrative nature of the review; however, study limitations and potential biases were considered during data interpretation.

RESULTS

LG (chemical formula $C_{27}H_{25}N_2NaO_7S_2$) is a diagnostic dye characterized by a green color that belongs to the xanthene family. LG exhibits high water solubility and demonstrates a peak absorption wavelength of around 624–635 nm, making it suitable for fluorescence and absorbance-based imaging techniques [6]. When instilled onto the ocular surface, LG selectively stains devitalized or damaged cells, enabling the visualization of epithelial defects, inflammatory changes, and irregularities [7]. As shown in Table 1, LG is a diagnostic dye similar to fluorescein but with different properties and distinct features.

LG staining is widely utilized in ophthalmic practice to assess ocular surface integrity and diagnose conditions such as DED and other ocular surface diseases [8]. Moreover, LG staining patterns can help identify disease etiology and provide a differential diagnosis based on location, intensity of staining, and amount of dye uptake.

Table 1 Summary of key features of LG, fluorescein, and rose bengal

Feature	LG	Fluorescein	Rose bengal
Type of dye	Non-fluorescent dye	Fluorescent dye	Non-fluorescent vital dye
Color	Green-blue	Yellow-green fluorescent	Pink-red
Chemical structure	Acid dye	Xanthene derivative	Oxanthene derivative
Main applications	Often used as an alternative to rose bengal for staining dead or damaged cells in the conjunctiva. Less irritating to the eye than other dyes and provides good differentiation between live and dead cells *Assessing cell viability, staining dead or damaged cells, ocular surface testing*	Particularly useful in ophthalmology for visualizing superficial lesions on the cornea and performing modified Schirmer's tests *Diagnosing and monitoring corneal erosions and abrasions, permeability tests, blood flow studies*	Stains dead or damaged epithelial cells and mucins; used in dry eye and herpetic keratitis
Tissues/cells stained	Dead or damaged cells, degenerative structures of the conjunctiva	Damaged epithelial tissues, ocular surfaces with epithelial dysfunction	Damaged cells, mucous strands, and exposed nuclei
Max absorption	Approximately 630 nm	Approximately 490 nm	Approximately 540 nm
Max emission	Not fluorescent	Approximately 520 nm (fluorescence)	Not fluorescent
Compatibility with other dyes	Often used with fluorescein, compatible with vital dyes such as phenol red	Often used with aniline blue, it can be used with lissamine green for comparative analysis	Incompatible with fluorescein due to interference

LG lissamine green

Lissamine Green and Dry Eye Disease

LG staining is used in the assessment and monitoring of DED, including its diagnostic patterns and correlation with disease severity. LG is especially useful in detecting conjunctival staining, a hallmark feature of DED associated with ocular surface inflammation and epithelial cell compromise. The recommendation for LG use in the 2007 Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) I report helped establish LG as an important dye in the armamentarium of tests employed to assess DED [9]. Its role has been further reinforced by the publication of the TFOS DEWS II Diagnostic Methodology report [2].

The staining pattern observed with LG varies depending on the severity and location of ocular surface damage. In mild cases, punctate staining is localized predominantly in the nasal and temporal bulbar conjunctiva. As the disease progresses, diffuse staining may spread across a larger portion of the conjunctiva, often extending to the corneal limbus. In advanced DED, dense, patchy staining patterns can be seen across the conjunctiva and corneal periphery, highlighting significant epithelial cell loss and inflammation. The differential staining patterns not only aid in diagnosing DED but also help evaluate disease severity, guide therapeutic interventions, and monitor treatment efficacy over time (Fig. 1) [10].

Inflammation Assessment

LG staining patterns have been linked to underlying inflammatory mechanisms on the ocular surface, supporting the use of LG as a marker of inflammation in ocular surface disease. The common denominator of pathological changes affecting the ocular surface is chronic inflammation [8]. It may result from tissue adaptation to prolonged stress conditions of various origins. A direct correlation between the degree of inflammation and the extent of LG staining has been proposed by Yang et al., who demonstrated that staining scores significantly correlate with the expression of interferon-γ, interleukin

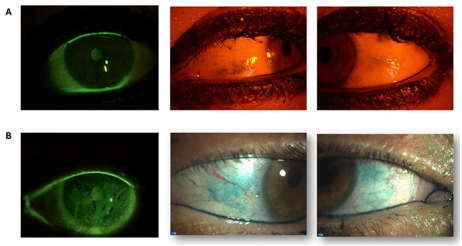

Fig. 1 Differential lissamine green staining patterns according to dry eye disease severity. **a** Moderate case of dry eye disease: negative corneal fluorescein staining (left), positive conjunctival lissamine green staining with red filter (right). **b** Severe case of dry eye disease. Corneal fluorescein staining (left), conjunctival lissamine green staining (right). Images from the personal archive of Prof. Barabino (Ocular Surface & Dry Eye Center, ASST Fatebenefratelli SACCO, Milan, Italy)

(IL)-6, IL-17, and matrix metalloproteinase-9 in Sjögren's syndrome (SS) and non-SS DED groups [11]. It is worth noting that correlation coefficients of all cytokines were substantially higher in SS DED than in non-SS DED. In addition, a pilot study reported a significant correlation between LG staining and $CD45^{+}CD14^{+}$ cell infiltration of the conjunctiva [12]. These data support the correlation between LG staining and the infiltration of immune cells in the conjunctiva.

Lissamine Green and Other Ocular Surface Diseases

Beyond DED, LG has diagnostic value in other ocular surface diseases, such as superior limbic keratoconjunctivitis and ocular cicatricial pemphigoid. Shiraishi et al. noted that LG staining was detected in more than 10% of patients with lid wiper epitheliopathy (LWE) and DED symptoms [13]. Moreover, the staining of the superior or inferior bulbar conjunctiva contributed to the differential diagnosis of ocular surface inflammatory diseases, e.g., superior limbic keratoconjunctivitis and conjunctivochalasis (Fig. 2) [5].

LG also plays a crucial role in the diagnosis and management of conjunctival cicatricial diseases, such as ocular cicatricial pemphigoid (OCP), a chronic autoimmune disorder leading

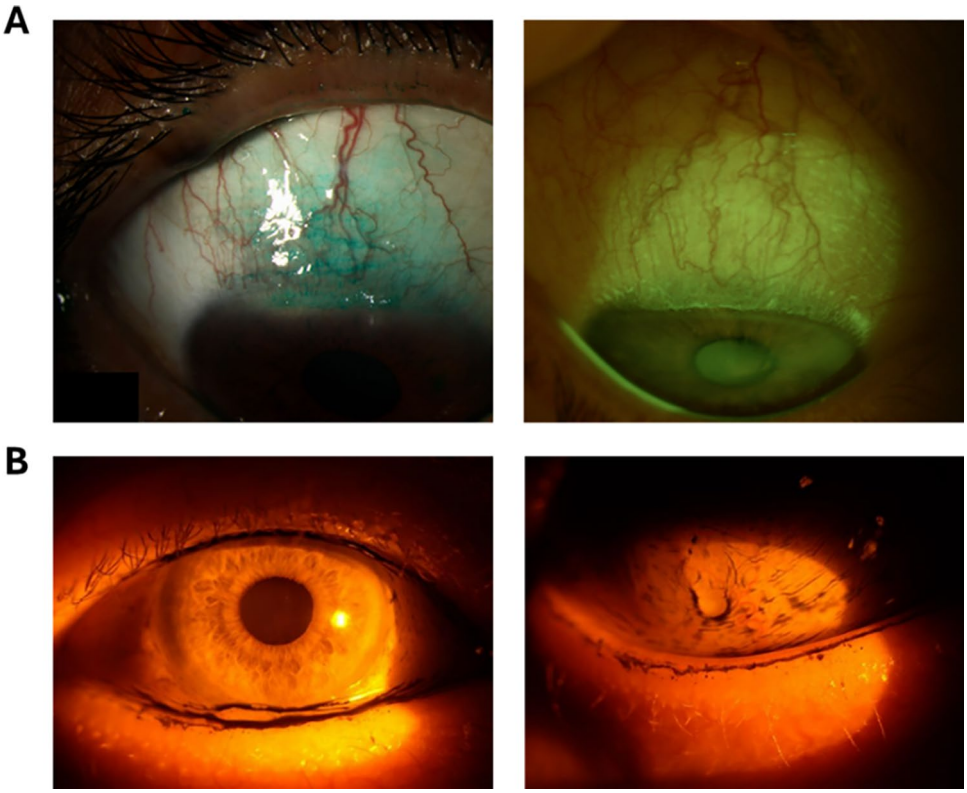

Fig. 2 Staining of the superior or inferior bulbar conjunctiva. **a** Superior limbic keratoconjunctivitis. Conjunctival damage was visible via lissamine green (left) or fluorescein (right) staining. **b** Conjunctivochalasis (left), irregular eyelid margin (right), lissamine green staining with enhanced contrast under red filter illumination. Images from the personal archive of Prof. Barabino (Ocular Surface & Dry Eye Center, ASST Fatebenefratelli SACCO, Milan, Italy)

to progressive conjunctival scarring [14]. LG is particularly useful in identifying areas of devitalized or necrotic epithelial cells, which are common in inflammatory and cicatricial conditions, such as OCP. The dye's ability to highlight damaged or missing epithelial cells helps clinicians assess the severity of conjunctival involvement, which is vital for monitoring disease progression.

In cases of OCP, the conjunctiva may show subclinical damage even before overt scarring is visible. LG staining can detect these early alterations in the conjunctival epithelium by revealing subtle staining patterns in the inferior and superior conjunctivas. These staining patterns may indicate early stages of inflammation and tissue damage, aiding in early diagnosis and timely initiation of treatment [15]. As the disease progresses and scarring worsens, LG staining patterns become more pronounced, typically highlighting areas of conjunctival keratinization, symblepharon formation, and shortening of the fornices.

Lissamine Green and Allergic Conjunctivitis

Allergic conjunctivitis (AC) presents unique staining features that can be captured using LG, in particular the vernal and atopic forms. AC is not a single disease but a collection of disorders with distinct signs and symptoms, phenotypes, prognosis, and management strategies. Epithelial barrier dysfunction contributes to the pathogenesis of AC [16], although epithelial defects are typical features of chronic forms, such as vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC). Several factors may contribute to the conjunctival and corneal epitheliopathy, including altered expression of

mucins and glycans, increased expression of proteases, cytokines, and chemokines, and the cytotoxic effect of eosinophil-derived granule proteins [17]. The evaluation of epithelial damage is important for assessing the severity of AC. LG staining can aid in identifying specific conjunctival changes characteristic of AC, such as corneal/conjunctival epitheliopathy, limbal Trantas dots, papillary hypertrophy, and follicular reaction. However, the Oxford and van Bijsterveld scoring systems used for DED do not seem adequate for assessing the epithelial damage in patients with VKC and AKC. Therefore, a specific staining score has been proposed [18]. The differences in staining patterns between limbal and tarsal VKC are particularly interesting. In limbal forms, the tips of active Trantas dots clearly stain with LG, while in active tarsal forms, the upper half of the cornea and conjunctiva stain with LG [18]. These findings can assist in distinguishing AC from other types of ocular surface diseases and guide appropriate management strategies.

Lissamine Green and Contact Lens

In contact lens (CL) practice, LG is used to assess ocular surface compatibility and identify complications such as LWE. In particular, LG is extensively used in CL practice, in both the prefitting assessment of ocular surface to investigate potential contraindications to CL wear and the follow-up of CL wearers to assess the interaction between CL and the ocular surface (CL-induced ocular surface staining) [19]. Usually, LG ocular surface staining in CL practice is quantified using grading scales [20, 21], although a digital grading of LG conjunctival staining has also been suggested [22–24]. It should be noted that detecting corneal staining with LG is less reliable than detecting conjunctival staining [25]. However, CL can cause conjunctival changes prior to any corneal changes [10]. For example, CL wearers with previous CL-associated red-eye episodes are more likely to exhibit a greater conjunctival response to CL than those with no previous inflammatory disease [26]. The symptoms described are very similar to those experienced by patients with DED, leading to this condition being termed CL-induced DED [27]. Therefore, LG can help define an individual's suitability for CL wear and guide recommendations for the most appropriate modality based on corneal and conjunctival staining [28, 29].

In CL aftercare visits, LG can help evaluate the interaction between CL and cornea, conjunctiva, and lid margin. The degree of LWE has been added to the list of clinical signs linked to CL discomfort [30–33]. LWE is characterized by epithelial abnormalities on the lid wiper region of the upper eyelid, which may result in upper lid margin staining. The primary cause of LWE is thought to be increased friction between the lid wiper and the ocular or anterior surface of the CL due to inadequate lubrication. This friction could be caused by DED and may be exacerbated by factors such as abnormal blinking patterns, poor CL surface lubricity, and adverse environmental influences [34]. More recently, it has been suggested that the etiology of LWE is multifactorial [35]. In this context, LG is used to reveal epithelial irregularities or abrasions on the lid wiper region [35, 36]. Korb graded LWE from 0 to 3 for each of two characteristics: the linear area of involvement and the severity of the staining [30]. LWE has also been classified into several morphologically distinct patterns [37]. More recently, a new photographic scale for LWE, the Photographic Lid Wiper Epitheliopathy (PLWE), has been introduced (Fig. 3) [38]. However, potential confounding factors must be considered, such as the number of eyelid eversions, which correlates with changes in the LWE pattern [39].

Liquid Lissamine Green: A New Formulation to Optimize Ocular Surface Staining

LG is now available in two different formulations. The most commonly used comprises impregnated paper strips containing 1.5 mg of the dye. A drop of sterile saline is added to the strip, and the dye is placed into the lower fornix of the eye by capillary action. However, given the importance of instilling an adequate volume of dye, it is worth noting that the LG in filter papers does not allow control over the amount of product released onto the ocular surface. Furthermore, the concentration of LG released by

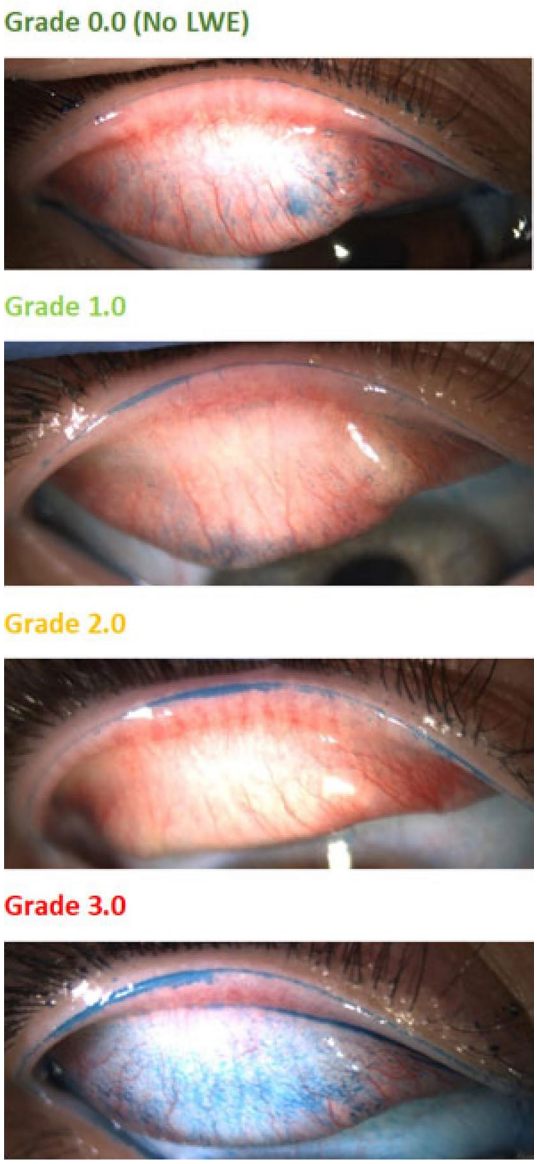

Fig. 3 The Photographic Lid Wiper Epitheliopathy grading scale (reproduced from Lievens CW, Norgett Y, Allen PM, et al. Development and validation of a new photographic scale to grade lid wiper epitheliopathy. Cont Lens Anterior Eye 2023;46:101773, published open access under the CC-BY-NC-ND license). The scale depicts increasing severity of epithelial damage on the lid wiper region based on both area and intensity of LG staining. This standardized tool supports objective assessment of LWE in clinical and research settings

filter papers is low and does not provide adequate staining. Recently, a new formulation of LG has been developed in liquid form, applied to the ocular surface by a patented dispenser to instill a calibrated drop of 10 µl (Lissafid, Fidia Farmaceutici, Italy). The LG concentration in the single-dose liquid formulation is 1.5%, and the vehicle is tamarind seed polysaccharide. The volume of 10 µl is based on a study that measured the optimal volume of LG for ocular surface examination in patients with DED [25], and obtained by a specific dispenser. The availability of this liquid formulation helps overcome the limitations associated with using LG strips, such as the need for dilution, the difficulty of calibrating the quantities of dye, and the coloring performance. The single-dose liquid LG has recently been used in a clinical study with optimal results [40]. The potential benefits in terms of staining consistency and ease of application are promising; however, they have not yet been confirmed through independent, head-to-head comparative trials. Further research is necessary to objectively evaluate the diagnostic accuracy and clinical outcomes associated with liquid LG versus traditional strip-based formulations.

To achieve optimum LG staining visualization, at least 30 s and no more than 5 min are required. An early visualization does not allow full development of staining patterns, and waiting too long may result in some of the pattern fading. The illumination level is also important: high illumination will bleach out some of the appearance of LG staining. Therefore, initial viewing should be performed under low illumination, which is gradually increased until the observation of any staining is optimal. Lastly, the contrast of the staining is enhanced if a red filter (567–634 nm) is used as a barrier filter on the slit lamp (Fig. 4) [20, 22, 25]. Recent advances in image analysis have also enabled the development of semiautomated and artificial intelligence (AI)-assisted approaches for evaluating LG staining. For instance, Bunya et al. reported a method for semiautomated quantification of conjunctival staining using digital image analysis [41]. These tools offer the potential for increased objectivity and reproducibility in LG evaluation. However, to date, no large-scale clinical trials have validated these AI-based

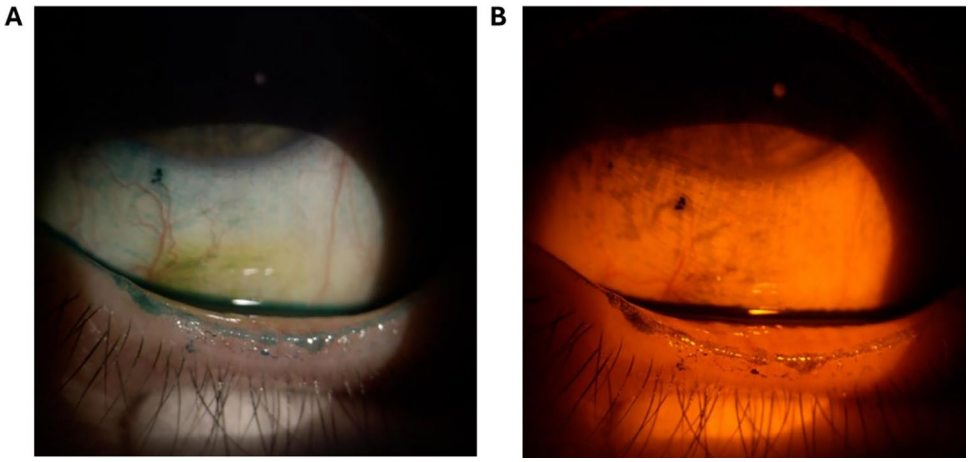

Fig. 4 Representative images of blepharitis, irregularities of Marx's line. Eyelid lissamine green staining with white light (**a**) or red filter (**b**). Images from the personal archive of Prof. Barabino (Ocular Surface & Dry Eye Center, ASST Fatebenefratelli SACCO, Milan, Italy)

diagnostic tools specifically for LG staining. Further research is needed to standardize these techniques and confirm their clinical utility.

Safety Considerations

Clinical trials evaluating the safety of LG in ophthalmic practice have reported minimal adverse events, including transient ocular surface discomfort or staining, which are generally mild and self-limiting [25, 42]. Chodosh et al. showed no significant toxicity or impact on corneal epithelial cell viability after exposure to LG for 5 min and subsequent washing with a buffer to mimic natural human tearing [43]. The analysis of corneal cells from five participants following as many as six instillations of 0.5% LG showed no significant increase in cell death [44]. A recent study investigated the performance of different LG strips on 22 volunteers. None of the participants reported discomfort, while the measured pH of the stained saline fell within the range that would not cause ocular surface burns or stinging [45]. Following LG application, routine rinsing is not necessary because of its low toxicity. However, excessive dye can be removed by blinking or gentle saline rinse if needed. Standard hygiene protocols should be followed to prevent cross-contamination. LG was found to be significantly more comfortable and less cytotoxic than rose bengal without compromising the quality of the ocular surface assessment. Furthermore, the duration of any discomfort was reported to be significantly shorter with LG than with rose bengal [46, 47]. The safety and tolerance of the new liquid formulation of LG have been confirmed by a clinical trial [40].

CONCLUSIONS

This narrative review aims at providing a comprehensive and clinically relevant summary of the current applications of LG in ophthalmology, including its diagnostic use across multiple ocular surface conditions, with emphasis on expert-driven clinical perspectives. Literature evidence and clinical experience suggest that LG represents a fundamental tool in ophthalmology, offering a non-invasive and effective means of assessing ocular surface integrity, diagnosing ocular surface disorders, and guiding therapeutic interventions. Its ability to selectively stain damaged conjunctival cells, combined with its favorable safety profile, makes it a first-line tool in clinical practice. Indeed, by providing detailed insights into the health of the ocular surface, LG aids in the effective diagnosis and

management of various ocular surface conditions, including DED, as well as in CL practice. Moreover, by facilitating the visualization of conjunctival changes associated with inflammation and allergic reactions, LG staining contributes to the assessment, diagnosis, and management of allergic eye disease while also helping manage and monitor ocular surface inflammation in DED. Its use, together with liquid fluorescein staining, provides a comprehensive view of ocular surface damage.

The recent availability of the single-dose liquid LG, equipped with a dispenser to instill a calibrated drop of 10 µl, makes its administration even easier during front-line visits. Thus, the use of liquid LG should be recommended as a routine assessment to improve and optimize the management of ocular surface conditions. Likewise, future research should aim to define standard procedures for the evaluation of LG staining by means of AI. It should be acknowledged that the narrative nature of the review introduces inherent methodological limitations. Unlike systematic reviews, this article did not apply structured appraisal tools or formal grading of evidence quality. Expert opinion from advisory board discussions was included to complement and reinforce published data but does not replace controlled comparative evidence. Future systematic reviews and meta-analyses are needed to further validate the clinical impact of the insights presented here.

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Data Availability. All data generated or analyzed in this review are included in this article and/or its figures. Further enquiries can be directed to the corresponding author.

Declarations

Conflict of Interest. Edoardo Villani received honoraria from Abbvie, Alcon, Bausch & Lomb, Diadema, Essilor Luxottica, FB Vision, Fidia, Santen, Sifi, Sun Pharma, Thea, Unifarco, Visufarma for contributing to advisory boards, educational events, focus groups and/or expert panels. Stefano Barabino, Pasquale Aragona, Stefano Bonini, Emilia Cantera, Antonio Di Zazzo, Giuseppe Giannaccare, Andrea Leonardi, Giancarlo Montani, Alessandro Mularoni, Vincenzo Orfeo, Fabrizio Zeri and Maurizio Rolando have nothing to disclose.

Ethical Approval. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors. All experts involved in the advisory board are authors of the paper.

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