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compare of performance of two brands of Lissamine green strips

The difference between two brands of Lissamine green dye to determining their staining ability in cornea, conjunctiva, Lid wiper and presenting Marx line

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Abstract

Background
Ophthalmic dyes are organic compounds widely used for diagnostic purposes. Lissamine Green (LG) is a vital dye with ability to stain ocular surface epithelial cells, unprotected by mucin or glycocalyx, also LG stains damaged cells. In European clinics GREEN GLO HUB LG strips were popular in past years but has recently been replaced by I-DEW GREEN strips. However, the staining ability of the new strips has been questioned. The purpose of this study was to compare the performance of two brands and in distinguishing staining of anterior surface and lid margins.

Methods
The cross over, randomized and comparative study was performed in 45 patients, in two consecutive days. One brand of LG was applied to one eye and the other brand to the other eye. The second day, the brands were switched to opposite eyes. Ocular surface disease index (OSDI) questionnaire was answered prior to the examination. The LG strips (1.5 mg concentration per strip) were diluted by a drop of sterile saline before a single controlled instillation in each eye. Two minutes after applying LG, OCULUS keratograph M5 was used for taking digital photos from nasal and temporal conjunctiva, cornea and the everted lower lids. The photos were then anonymized before grading of corneal and conjunctival staining and grading of lid wiper epitheliopathy (LWE). Marx line appearance was recorded as present or absent. Burning sensation was recorded from 0(no sensation) to 3(severe sensation) based on the patients' subjective opinion.

Results
In this study, there is a significant difference in ocular surface staining between the two brands of LG p=2.46 e-05 ($p < 0.001$). The mean value for Oxford grading of the ocular surface for LG1 was 0.74 and for LG2 was 0.046. In grading of the LWE for LG1 27.89% had grade 1 or above. For LG2 none of the photos showed any LWE, so all of them graded as 0. Also, none of the photos with LG2 showed Marx line, whereas 60.46% showed Marx line with LG1. In subjective reporting of burning sensation there was no significant mean difference between the two brands of LG (p=0.4309).

Discussion
This study showed that there is significant difference in staining performance between two brands. The results show higher mean scores in Oxford grading for LG1 compared to LG2. These results might prove weak performance of LG2 in staining of ocular surface. Also, LG2 didn't show LWE and Marx line in any of the photos, whereas LG1 showed LWE and Marx line in a significant number of photos. Due to no LWE and absence of Marx line in all cases related to LG2, it seems this brand is unable to be the proper diagnostic dye in detecting eye structures' changes, especially lid margin changes.

Conclusion
There was significant difference in performance between two brands of LG in Oxford staining, LWE and Marx line showing up. There was no difference in reporting burning sensation between the two LG brands.

Key words: Lissamine Green, corneal and conjunctival staining, LWE, Marx line, Burning sensation

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Foreword

This thesis is my final work of my master study in general practice optometry University of South-Eastern Norway. I did this research in Covid-19 situation regardless of limitations of quarantine in clinical environments, and during that time the only encouragement for me was accompanying my teacher, Ann, who believed in my work.

I hope this report will be beneficial for ophthalmic practitioners and would be a small step forward improving the knowledge of humanity.

I would like to express my appreciation to Morteza Hajihosseini, Epidemiology PHD candidate, school of Public Health, University of Alberta, for his helpful statistical contributions.

I would like to thank my brothers for their permanent support,
And thank my mother for being.

1 Introduction

In many of the ocular surface conditions such as dry eye disease (DED), punctate staining of ocular surface is one of the main features, and ophthalmic dyes are used widely in diagnosis and management of these conditions. In clinical ophthalmology, dyes like sodium fluorescein (FL), Rose Bengal (RB) and Lissamine green (LG), among other dyes, are the most frequently used dyes (Kim, 2000).
A dye is an organic compound and it has two chemical groups, one chromophore group and other one auxochrome group attaching to a benzene ring (Kim, 2000). If a dye can stain living cells and tissue it is called vital dye (Feenstra & Tseng, 1992). The use of vital dyes first introduced in 1882, when sodium fluorescein was known as having detected breaks in corneal epithelium continuity (Campbell & Boyd, 1950).
The ocular surface staining with using sodium fluorescein appears when viable cells lose their integrity, such as disruption in superficial cell junctions or when cells experience Glycocalyx defection on their surface (Bron, Argüeso, Irkec, & Bright, 2015). FL stains healthy cells, dead cells, damaged cells and intracellular spaces (https://www.opticianonline.net/cet-archive/4925).
Rose Bengal (RB) is a derivative of fluorescein has been widely used since its first reported use in 1914 (Norn, 1970). RB stains ocular surface epithelial cells which are not protected by mucin or glycocalyx. This dye detects dead or degenerated cells as well (Khan-Lim & Berry, 2004).
Additionally, it has been proved that RB suppresses human corneal epithelial cell viability in vitro (Manning, Wehrly, & Foulks, 1995). Rose Bengal is used seldomly now, because it stings on instillation and induces reflex tearing.

The dye which has a nearly identical staining profile as Rose Bengal, has been used since 1973, without discomfort and toxic effect, is Lissamine green which gives better patient tolerance than RB (Bowling, 2005; Manning et al., 1995). LG is a synthetically produced organic acid dye with alternative synonyms of acid green, wool green S or C, and fast light green. LG stains ocular surface epithelial cells which are unprotected by mucin or glycocalyx, also damaged cells as well as Rose Bengal (Kim, 2000; Manning et al., 1995).
RB stains dead or degenerated cells (Norn, 1970), also healthy cells (McDonnell, (2010)), therefore LG has a fundamental rule in distinguishing damaged cells in Dry eye condition because it doesn't stain healthy cells. It is agreed that LG is much effective in assessing conjunctival staining than corneal integrity assessment and the reason is poor visibility in dark iris background and marked corneal staining with LG is only seen in patients with Sjogren type dry eye (McDonnell, (2010)). In evaluating ocular surface staining with LG, a red filter (567-634 nm) is needed to enhance good staining visibility

(P. Hamrah, F. Alipour, S. Jiang, J. Sohn, & G. Foulks, 2011). Research are ongoing to find what LG stains exactly.

In ocular surface field the lid wiper region is becoming an area of intrest.The lid wiper is that portion of the marginal conjunctiva of the eyelids which act as a wiping mechanism for spreading the tear film over the ocular surface or the surface of a contact lens. The act of spreading the tear film is mostly in relation with upper lid margin and lower lid wiper has less role. Lid wiper epitheliopathy (LWE) is the term which describes the disruption to the surface epithelium of the lid wiper, and it is mostly observed while using ophthalmic dyes (Knop et al., 2011). Studies showed that the prevalence of LWE is greater for dry eye patients and could be used as a diagnostic sign of dry eye disease (Korb et al., 2010). Also, in contact lens users LWE is more visible compared to control groups.Another lid margin structure which could be seen by stains is Marx lin. It is a distinct line of cells between the eyelid margin and bulbar surface and first time was defined as mucocutaneous junction by Marx in 1924. This line provides information about meibomian glands disfunction (Yamaguchi et al., 2006). Compared to F and RB, LG is better choice when it comes to looking for lid margin changes such as lid wiper epitheliopathy (LWE) because of contrast of this dye's colour in front of eye's structures colour, also due to visibility of Marx's line, makes LG more appropriate dye in investigating of chronic meibomian gland dysfunction (McDonnell, (2010)).

In clinics the most popular using form of LG is strips which should be moist with saline but in research for controlling the quantity better, solutions (in 1%, 2%, 3%) are used (https://www.opticianonline.net/cet-archive/4925). Also, studies show that different brands slightly vary in staining quality and different manufacturers strips have variation in clinical performance (Delaveris, Stahl, Madigan, & Jalbert, 2018). In European clinics GREEN GLO HUB LG strips were popular in past years but recently, I-DEW GREEN strips has got the CE approval to be used in clinics, but it seems the new strips doesn't have good efficiency in determining ocular surface staining. In this study we will try to compare the performance of two brands and their utility in distinguishing staining of different parts of eye surface.

In TFOS DEWS II definition for dry eye, there are sing and symptoms related to the ocular surface. Although, the relationship between clinical signs and symptoms are not linear but quantifying the ocular surface symptoms is still important tool for screening and establishing medical management for DED cases. For this matter, at the beginning of the patient examination a validated symptom questionnaire is recommended. OSDI (Ocular Surface Disease Index) questionnaire is the most widely

used for DED clinical trials (James S. Wolffsohn et al., 2017). The OSDI includes 6 questions related to visual disturbance (blurred vision or poor vision), visual function (problems in reading, driving at night, working on a computer or watching TV).

For recording of ocular surface staining there are various grading systems including Bijsterveld system, the National Eye Institute/Industry Workshop guidelines, the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) schema, Oxford Schema, the area-density combination index and Sjogren's International Collaborative Clinical Alliance ocular staining score (James S. Wolffsohn et al., 2017).

2 Issue

Research objectives and significance:

The main purpose with this study is to compare the performance of two different brands of Lissamine green dye strips with the same amount of dye concentration (1.5 mg per strip):

The main objective of this study was based on following questions:

A. Is there any significant difference in staining scores based on Oxford grading system for corneal and conjunctiva, when we use two different brands of LG?

B. Is there any significant staining difference in lid wiper based on ULMS eye dryness classification scale when we use two different brands of LG?

C. Is there any difference in ability of showing Marx line between two brands of LG?

D. Is there any difference of patient subjective reporting of burning sensation between two types of LG using in study?

The secondary objectives in this study is based on following questions:

A. Is there any correlation between age and staining scores with two types of LG?

B. How strong is the association between OSDI scores and staining scores with two types of LG?

C. Is there any correlation between gender and staining scores with two types of LG?

Based on these objectives we formulated following hypotheses:

For corneal and conjunctival staining scores according OXFORD grading system:

H0: There is no significant difference between two LG brands staining scores

H1: There is significant difference between two LG brands staining scores

For lid wiper staining scores according to ULMS eye dryness classification scale:

H0: There is no significant difference between two LG brands staining scores

H1: There is significant difference between two LG brands staining scores

For Marx line presence:

H0: There is no significant difference between two LG brands in showing Marx line

H1: There is significant difference between two LG brands in showing Marx line

For burning sensation:

H0: There is no significant difference of reporting burning sensation between two LG brands

H1: There is significant difference of reporting burning sensation between two LG brands

The results are expected to improve our knowledge about performance of two different old and new brands of LG used by clinicians for evaluating ocular surface integrity.

3 Methods

The type of research was cross over, randomized and experimental design for comparing functional ability of two brands of Lissamine Green strips in staining anterior surface of eye and lid margins.

3.1 Participants

Subjects were recruited from students and faculty in University of South-Eastern Norway and Norwegian Language center of Kongsberg in one month (during August 2020), by inviting students in their classes and sending e-mail to teachers. For increasing attendance rate, we were reminding subjects by verbal notice for participating in the examination. Test were performed in National center of optics, vision and eye care in University of Southeast Norway, Kongsberg.

With approval of the Norwegian center for research data (NSD), 45 subjects with various ethnic, (32 females and 13 males), aged 18 to 63 (mean (Sd) 30.82 ±11.2) at the time of examination participated in this study. The participants had ethnic diversity from African, Asian and European countries. Inclusion criteria were, normal healthy individuals with dry eye or without dry eye, anterior blepharitis, contact lens (CL) users, eye drop users and subjects with eye allergy history. Exclusive criteria were participants with eye infection and eye allergies in which invasive methods might irritate the eyes more. Subjects with contact lenses, who didn't want to bring out their lenses for any reason didn't enter to the study as well.

3.2 Apparatus

All equipment was available in university's optometry clinic, USN, Kongsberg, Norway:
Two brands of Lissamine green:
1)GREEN GLO HUB strips (in our study **Brand 1**, **LG1**), Ref: OLPL/HUB/LBL/V7-01, Batch No: HUL0001, each strip is impregnated with 1.5 mg of LG, manufactured by: Omni Lens Pvt. Ltd, Expiry date: Sept 2024

2)I-DEW GREEN strips (in our study **Brand 2, LG2**), CE (0068) approval, Batch No: ERC/A3/002, each strip is impregnated with 1.5 mg of LG, manufactured by: Entod Research Cell UK. Ltd, Expiry date: JAN 2022

-Sterile saline liquid POLYRINSE-U, manufactured by Alcon Laboratories Inc,19272A

-OCULUS keratograph 5M, Typ 77000, SN 3711 2190 24V DC 2, 1A, No.05150150

-Cotton soaps

-Incidin OxyWipe S, surface cleaning and disinfection wipe (for medical devices)

-Face masks

-Staining grading systems:

- Grading of corneal and conjunctiva staining Oxford scheme (Appendix 1)
- Pictures of ULMS eye dryness classification scale by Dr Elena Garcia Rubio (Appendix 2)

-Ocular Surface Disease Index (OSDI questionnaire) (Appendix 3)

-Consent forms by NSD (Appendix 4)

-Personal laptop, word, Excel and data processing R commander program, portable hard disk

3.3 Procedure

Examination was performed on two separate and consecutive days, preferably at the same time on both days, first day brand 1 was used in one eye and brand 2 in the other eye, and second day opposite brands were used in each eye. Choosing LG brand for each eye on first day was randomly and we used both brands in first day for patients. Subjects were requested to arrive to the clinic between 9:00 and 16:00 in each day. In this study there were separate recording system for grading corneal, nasal conjunctive and temporal conjunctive staining, and lower Lid wiper epitheliopathy (LWE) for both dyes. We did not consider upper lid wiper epitheliopathy.

Because of Corona situation during collecting the data in clinic (August 2020), we had special measurements in order to prevent infection spreading. We provided consent forms, OSDI questionnaires and a form related to Corona virus questions before patients come to the clinic and they all brought forms filled. Before entering to the clinic for each patient we disinfected in used equipment and other surfaces which were in contact with patient and examiner by Incidin OxyWipes S, and both examiner and patient were wearing face masks while being in clinic. The examiner took Covid-19 virus prevention course in early August, at USN before starting examination.

All subjects have been registered in OCULUS keratography M5 with their first name, last name, date of birth and an ID number before starting the tests. A ready examination form was available for recording all subjects' information, plus date of using each brand in each eye to be able to find the correct dye and eye on following day, burning sensation related to each brand and eye, using of contact lenses or drops, and having allergy.
As concentration of LG, volume of instillation and duration of contacting with eye structures are important factors for making reliable decision (Foulks, 2003; Yerxa et al., 2002), we tried equal application of two brands of LG in all tests. Both brands of LG strips we used in the study had 1.5 mg concentration of dye per strip. One drop of Sterile saline liquid POLYRINSE-U used to wet the strips (McDonnell, (2010)). After 5 seconds waiting for eluting the dye by saline (J. S. Wolffsohn et al., 2017), we did a single shake to remove extra eluted dye from the strips.

We asked patients to look up and nasally while each application of dye and lower eyelids pulled slightly down from temporal side. We tried to avoid touching the lower lid wiper while pulling the lid down, for preventing unwanted fingerprints and other marks which might be caused by external touch. We applied the LG strips to temporal lower conjunctiva one time, and asked patients to blink gently to allow adequate staining for whole eye surface (McDonnell, (2010)). Examiner used right hand when applying LG in Left eye of the patient, and left hand to apply the dye in patients' right eye to avoid any damage to the cornea. We didn't use any anesthetic during the LG instillation.

According to the studies, timing and duration of exposing eye structures to LG dye is an important factor (Kim, 2000). Therefore, for achieving the right amount of staining in each eye, we tried to record images within 2 min post instillation of LG in conjunctive and cornea. For assessing lid wiper epitheliopathy recommended timing is 3-6 min after instillation (J. S. Wolffsohn et al., 2017), for this matter we pulled lower lids for recording LWE images after conjunctiva and corneal images recording. After finishing with the first eye and first brand of LG, we did the same for the other eye and other LG brand. OCULUS keratograph M5 (a digital camera with a stationary computer which is not linked to any network) used to take pictures of subjects' eye. in this instrument, 1.00 magnification used for corneal, nasal and temporal conjunctive staining and 0.5 magnification used for lower LWE. On the following day, we switched the LG brands between two eyes to make sure about reliability of the test and comparing each eye with two different brands of the LG. Since we used two brands in each eye with at least 24 hours interval, this period is proper timing for washing out the first LG brand, and it does not affect the second brand's staining grade on the second day of recording.

In order to keep subjects' information anonymous, all taken photos downloaded to a portable hard disk with new ID codes without patients' names and name of the LG brand. Then photos uploaded in Teams-wind in university website, in which examiner and teachers could have access to them anonymously. One examiner(student) graded the photos.

For assessing corneal and conjunctival staining, we used Oxford grading system (Appendix 1). This grading scale is mostly used with ophthalmic dyes assessments, which divides outer eye staining into six degree according to the severity, from 0 (absent) to 5 (severe). With this system examiners compare the overall appearance of the cornea and conjunctival staining with reference figure without any attempt to count the dots or assess the place of the dots. For each eye there are 3 zones, nasal conjunctiva, cornea and temporal conjunctiva. Therefore, in maximum severity of staining (score 5 for each zone), the maximum score for an eye would be 15.

For LW assessment we used pictures of ULMS eye dryness classification scale by Dr Elena Garcia Rubio (Appendix 2). It is also a reference figure in which LWE has 5 staining category ,
No staining=0 15% staining = 1 25% staining= 2 50% staining=3 100% staining=4.
In this grading, we compared taken lid wiper pictures to ready figures, and graded them without measuring the sagittal width and length of LWE (James S. Wolffsohn et al., 2017).
For recording Marx line, we described two nominal codes where present Marx line recorded as (1), and absent Marx line recorded as (0). Also, in order to compare burning sensation between two brands of LG, in examination forms we defined ordinal subjective scales, in which no sensation=0 slight sensation=1 moderate sensation =2 severe sensation=3 based on patients' subjective opinion.
The new ID codes and staining grades punched in Excel data sheets. Afterwards, the first row Excel was sent to supervisor to add information such as age, gender, OSDI score, LG brand and other information related to each grading. Then the final excel was ready for final data modifying and statistical analysis. We sorted all LG1 scores in one column and all LG2 scores in other column without considering left and right eye and for each brand we had 86 samples (considering 4 missing data for both brands).

3.4 Data analysis

Variables are described as mean (SD) or count (percentage) as appropriate. Two independent samples t-test was used for the first objective compare Oxford grading system for corneal and conjunctiva between two different brands of LG. For categorical outcomes such as LWE, Marx-Line, and Burning sensation chi-square test was used to compare two different brands of LG. Linear and logistic (binary and multinomial) univariate regressions were used for the secondary objectives to test the effect of other variables such as age, gender, and OSDI on the continuous and categorical outcome variables. For some variables, the algorithm did not converge, so that the data are described without statistical significance testing. The analyses were conducted using R software version 4.0.0 and P-value of ≤ 0.05 was used to determine statistical significance.

4 Results

Forty-five participants aged between 18 to 68 entered the study. Between those, 3 participants did not join examination on the second day and for 1 of the subjects we had wrong picture recording on the second day. In statistical analysis with R programme they were considered as missing data (NA). Also, four participants had tearing while applying LG, but results presented from all subjects, including these four cases. 71.1 % of the participants were and 28.8% of the subjects were male. Five of the subjects were contact lens users, 5 of them reported eye allergy history and 5 of them reported using eyedrops (Table 1).

Table 1:descriptive results

Age **Mean (Sd) (y)**	30.82 ±11.2
Gender **N (%)**	F: 32 (71.1 %) M:13 (28.8 %)
OSDI **Mean (Sd)**	20.19 ±16.7
Allergy **(%)**	(11.11 %)
Eyedrop users **(%)**	(11.11 %)
CL wear **(%)**	(11.11 %)

In comparing the performance of the two LG brands, the results showed significant difference between two brands when evaluating corneal and conjunctival staining based on Oxford staining

grading. The mean score of Oxford grading for LG1 was significantly higher than LG2 mean score (0.74 ± 1.41 versus 0.046 ± 0.26). LG2 did not present LWE and Marx line in any of the subjects, and 100% of cases had no staining for LWE and Marx lines were absent with LG2. However, LG1 showed big difference for these two variables compared to LG2 (P=3.827e-06 for LWE difference and P=2.2e-16 for Marx line difference). There was no significant difference in burning sensation between two brands of LG. None of the subjects reported severe burning sensation with two brands of the LG (Table2).

Table 2:Descriptive results

Table **Variable**	Groups		P-value
	LG1	**LG2**	
OXFORD Mean (Sd)	0.74 ± 1.41	0.046 ± 0.26	P=2.46e-05 (P<0.001) **
LWE N (%)			
No staining (0)	62 (72.09 %)	86 (100 %)	P=3.827e-06 ** (P<0.001)
15% staining (1)	20 (23.25 %)	0%	
25% staining (2)	3 (3.48 %)	0%	
50% staining (3)	0%	0%	
100% staining (4)	1 (1.16 %)	0%	
MARX-LINE N (%)			
Absent Marx line (0)	34 (39.53%)	86 (100%)	P=2.2e-16 ** (P<0.001)
Present Marx line (1)	52 (60 .46%)	0%	
BURNING SENSATION (%)			
No sensation (0)	39 (44.82%)	38 (43.67%)	P=0.43
Slight sensation (1)	42 (48.27%)	38 (43.67 %)	
Moderate sensation (2)	6 (6.89%)	11 (12.64%)	
Severe sensation (3)	0%	0%	

Linear regression analysis showed that variables such as age, gender and OSDI did not have association with Oxford scores. Also, for each group of LG brands separately these variables did not have significant effect on Oxford scores of LG1 and LG2 statistically. According to the regression results with increasing one year of age, Oxford scores will increase only 0.025 and 0.001 with using LG1 and LG2 respectively. With increasing one unit of OSDI scores, Oxford value decreased for

both brands almost similarly, and for gender, different pattern of changes in Oxford scores happened for each brand of LG by changing gender from male to female (Table 3,4,5).

Table 3:Linear Regression for Oxford Grading

Variable	Coefficient	SE	T-value	95%CI	P-value
AGE (y)	0.013	0.0071	1.91	[-0.0003, 027]	0.056
OSDI	-0.0026	0.005	-0.52	[-0.012, 0.007]	0.6
GENDER (M)	-0.13	0.17	-0.77	[-0.49, 0.21]	0.43

Table 4:Linear Regression for Oxford Grading in LG1

Variable	Coefficient	SE	T-value	95%CI	P-value
AGE (y)	0.025	0.01	1.94	[-0, 0.05]	0.54
OSDI	-0.003	0.009	-0.34	[-0.022, 0.015]	0.72
GENDER (M)	-0.31	0.33	-0.92	[-0.98, 0.35]	0.35

Table 5:Linear Regression for Oxford Grading in LG2

Variable	Coefficient	SE	T-value	95%CI	P-value
AGE (y)	0.001	0.002	0.52	[-0.003, 0.006]	0.6
OSDI	-0.001	0.001	-1.09	[-0.004, 0.001]	0.27
GENDER (M)	0.043	0.06	0.7	[-0.07, 0.16]	0.48

For LWE staining we had 5 categorical scales, and 4 categories of 5 had frequency in our results. With considering grade 0 (no staining) as reference, and assessing the correlation of the age, OSDI and gender on LWE scores, we only found significant impact of age on grade 1(15% staining) of LWE. It means with increasing one unit of age, LWE score for grade 1 of staining, will increase 6% compared to first category of staining (0% staining) (Table 6).

In Marx line presence, only gender had significant p-value and with considering male as reference this showed that the chance of occurring Marx line in male gender is 2.2 times compared to female gender (Table7).

Age, OSDI and gender variables did not show significant correlation with burning sensation subjective report. Also, multinomial regression for each brand of LG separately, did not show any regression between age, OSDI and gender variables and burning sensation degrees (Table 8).

Table 6:Multinomial logistic regression, LWE

Variable	Estimate	SE	Statistic	95%CI	P-value
Age(Y)					
0	Reference	-	-	-	-
1	1.06	0.019	3.122	[1.022, 1.1]	0.002**
2	0.944	0.08	-0.724	[0.807, 1.104]	0.469
4	1.001	0.094	0.01	[0.832, 1.204]	0.992
OSDI					
0	Reference	-	-	-	-
1	0.993	0.015	-0.445	[0.965, 1.023]	0.656
2	0.87	0.104	-1.333	[0.710, 1.067]	0.183
4	0.912	0.126	-0.731	[0.712, 1.168]	0.465
GENDER(M)					
0	Reference	-	-	-	-
1	1.74	0.492	1.125	[0.663, 4.56e+0]	0.26
2	5.215	1.237	1.335	[0.462, 5.89e+01]	1.182
4	3312.99	35.588	0.228	[0, 6.50e+33]	0.82

Table 7:Binary Logistic Regression, Marx line

Variable	Estimate	SE	statistic	95% CI	P-value
AGE(Y)					
0	Reference	-	-	-	-
1	1.013	0.014	0.893	[0.984, 1.041]	0.372
OSDI					
0	Reference	-	-	-	-
1	0.99	0.01	-0.981	[0.969, 1.01]	0.326
GENDER(M)					

0	Reference	-	-	-	-
1	2.2	0.351	2.246	[1.103, 4.389]	0.025**

Table 8:Multinomial Logistic Regression, Burning Sensation for LG

Variable	Estimate	SE	statistic	95% CI	P-value
AGE(Y)					
0	Reference	-	-	-	-
1	1.007	0.014	0.452	[0.979, 1.035]	0.651
2	1.029	0.022	1.289	[0.985, 1.074]	0.197
OSDI					
0	Reference	-	-	-	-
1	0.986	0.01	-1.429	[0.968, 1.005]	0.153
2	0.987	0.017	-0.763	[0.954, 1.021]	0.446
GENDER(M)					
0	Reference	-	-	-	-
1	1.264	0.342	0.686	[0.647, 2.471]	0.493
2	0.147	1.06	-1.81	[0.018, 1.173]	0.07

Table 9:Multinomial Logistic Regression, Burning Sensation for LG1

Variable	Estimate	SE	statistic	95% CI	P-value
AGE(Y)					
0	Reference	-	-	-	-
1	0.998	0.02	-0.09	[0.961, 1.037]	0.928
2	0.986	0.041	-0.342	[0.909, 1.069]	0.0.732
OSDI					
0	Reference	-	-	-	-
1	0.974	0.016	-1.017	[0.898, 1.151]	0.286
2	1.01	0.017	0.19	[0.8, 1.96]	0.902
GENDER(M)					
0	Reference	-	-	-	-

1	1.495	0.342	0.189	[0.352, 2.45]	0.702
2	0	20.95	-0.42	[0, 3.604]	0.808

Table 10:Multinomial Logistic Regression, Burning Sensation for LG2

Variable	Estimate	SE	statistic	95% CI	P-value
AGE(Y)					
0	Reference	-	-	-	-
1	1.01	0.022	0.753	[0.974, 1.06]	0.451
2	1.054	0.028	1.869	[0.997, 1.115]	0.062
OSDI					
0	Reference	-	-	-	-
1	0.984	0.014	-1.157	[0.958, 1.011]	0.247
2	1.005	0.027	0.198	[0.953, 1.06]	0.843
GENDER(M)					
0	Reference	-	-	-	-
1	1.125	0.477	0.247	[0.442, 2.865]	0.805
2	0	38.95	-0.213	[0, 3.54]	0.831

Note: During examination, we found different behavior between two brands of the LG while wetting with saline. LG1 retained the saline drop concentrated in the place of drip until shaking the strip and pushing the saline drop to wet whole strip. For LG2 whole drop went through the strip and wet it immediately after trickle the saline drop (Figure 2,3). There was a slight difference in thickness of the strips as well and LG1 had thicker strips compared to the LG2 brand strips.

Note: While applying LG brands in conjunctiva and photography with OCULUS M5, the place of touching with LG1 strip had sharp color with LG1 and in a few cases, it was difficult to differentiate with ocular surface staining. However, for LG2 the place of applying the strips were without color while taking pictures.

5 Discussion

Several dyes are in use for diagnostic purposes in ophthalmic clinics. Sodium fluorescein, Rose Bengal (RB) and Lissamine Green (LG) are most common dyes. Sodium fluorescein was known as premier dye for corneal staining and RB for conjunctival staining. Even though, RB is still one of the most commonly used stains (together with fluorescein), but studies showed its toxicity to cell membranes, including discomfort feeling to the patients and decreasing the chance of recovery of herpes viruses in human cell cultures (Chodosh, Banks, & Stroop, 1992). Previous researches have established that RB stains dead and damaged cells, but recently studies proved that RB adversely affects human corneal epithelial (HCE) cells viability and stains normal proliferation cells too (Kim & Foulks, 1999). Because of these facts, LG which has almost same identical feature for detecting ocular surface staining as RB, could be proper replacement for RB in clinics. When it comes to assessment of conjunctival staining, LG might be better choice because of its bluish green colour and its better contrast on the conjunctiva. Likewise, compared to FL and RB, LG is better choice when it comes to observing lid margin changes such as lid wiper epitheliopathy (LWE) because of contrast of this dye's colour in front of eye's structures colour. Similarly, due to the visibility of Marx's line with LG, this stain becomes more appropriate dye in investigating of chronic meibomian gland dysfunctions (McDonnell, (2010)). With this in mind, we can conclude the importance of using LG in investigating ocular surface abnormalities and its place in clinical usage in recent decades.

The popular type of LG stain for clinical investigations is solutions (in 1%, 2%, 3% concentrations), because in solutions it is easier to control their usage amount. However, in clinics, practitioners usually don't make solutions and they use LG strips by wetting them with saline. Almost all LG strips have 1.5 mg concentration on each strip, but it seems there is variation of efficiency between different brands. There are several studies which are comparing the different brands of LG performance and in this study we tried to investigate the difference of two brands which one is recently used ,I-DEW GREEN (LG2), and the other brand GREEN GLO HUB (LG1) which is not in use in European clinics any further.

For this purpose, we needed to perform the exact method for applying each brand. As the applied quantity from a strip can vary, and this could affect the amount of staining and comparison results, we took all examinations in the similar way as possible to minimize variability of testing. Because of this fact, timing, duration of exposure of LGs to the eye structures and volume of instillation considered equally for both brands. Our goal was to compare two brands of LG in the both eyes of

the same patients (crossover study), by switching the dye brands on two consecutive days between left and right eyes of a patient. We tried to perform the test at the same time on first and second days for each patient, to prevent ocular surface alteration of patients' eyes during the daily activities.

We included different age groups in the study (aged 18 to 63); as many literatures show there is a positive association between aging and clinical marks of dry eye such as LWE, meibomian gland dysfunction and ocular surface staining (Wang et al., 2020), these changes in aged people eyes could be helpful in finding marked differences between two LG brands. In this study we determined dry eye disease severity by the Ocular Surface Disease index (OSDI questionnaires) and tried to find OSDI score association with staining levels from two LG brands. Previously, studies proved that dry eye diseases (DED) and consequently its clinical signs occur more frequently in females than males (Sullivan et al., 2017), so gender was considered as other variable which was important to know its association with LG performance. Included in the study were patients with various ethnic origin from Asia, Africa and Norway. Even though, research has proved that different ethnics have different staining scores with ophthalmic dyes, in this study we didn't take ethnic variable into consideration and for this kind of investigation we would probably need larger sample population.

According to the studies, timing and duration of exposure of the eye structures with LG is important factor in staining amount (Kim, 2000). Researchers have come to a conclusion that the optimal time for evaluation conjunctival staining is between 1 to 4 minutes after applying LG dye (Kim, 2000; McDonnell, (2010)). In other study, optimal viewing condition was suggested to be within 2 minutes post instillation (McGinnigle, Naroo, & Eperjesi, 2012), because intensity of the dye can diminish after two minutes. Instant viewing could result in wrong interpretation due to not enough dispersion of the dye in the outer eye surface (Foulks, 2003). Also, for LWE recommended viewing time is 3-6 min post instillation. For controlling the timing factor, we waited for 2 minutes after each application of the stains and captured conjunctival and corneal pictures, and after that we tried LWE and Marx line pictures recording which was automatically between 3 and 6 minutes after instillation.

As cited before, LG is much effective when it comes to the conjunctival staining, but in the corneal integrity assessment because of the poor visibility in dark iris background it is hard to see LG staining. For optimal assessing of the corneal staining with LG, white light of slit lamp and a red transmitting filter (for example Hoya 25A or Kodak Wratten 92) is needed (P. Hamrah, F. Alipour, S. Jiang, J. H. Sohn, & G. N. Foulks, 2011). In current study, because of the Covid-19 limitations, we only considered photos which taken with OCULUS keratograph 5M for both corneal and

conjunctival staining and we didn't observe corneal staining through the slit lamp and red filter. This might affect the staining score in general and underestimate it. However, since this method applied for both LG brands in the same standard technique it will not affect the comparing results.

There are several systems for grading ocular surface staining in clinical use, Van Bijsterveld system, Oxford system and standardized version of the NEI/CLEK system are three important systems. Oxford and CLEK systems use a wider range of scores allowing for the detection of smaller steps of changes in clinical trial, and CLEK system evaluates several zones of cornea, including visual axis. No studies indicated that one grading system to be better than others (James S. Wolffsohn et al., 2017).

In this study for grading our subjects' ocular surface staining we used Oxford system.

As cited in past paragraphs, in Oxford scheme each eye surface has three zones, nasal conjunctiva, temporal conjunctiva and corneal part. For each zone it has staining grade from 0 to 5, and in minimum state of staining there is total 0 score for three zone, and in maximum staining condition there is total score of 15 for each eye. We compared taken photos from subjects' eye surface with reference Oxford figures without counting the staining dots. In light coloured irises corneal staining is a little more visible than brown irises and in general significant corneal staining with LG is only seen when patients has Sjogren's type dry eye (McDonnell, (2010)), and in this study we didn't have Sjogren's type dry eye and a few visible corneal staining that we observed had low scores compared to the conjunctival staining scores.

LWE results from inadequate lubrication and decreased tear film thickness at the lid margin which could be associated with dry eye condition or contact lens (CL) wear. Decreased lubrication causes friction and mechanical trauma to epithelial cells during blinking, and lid margin rubs against corneal surface and make epithelial cells resized and reshaped (Efron, Brennan, Morgan, & Wilson, 2016; Korb et al., 2005). As mentioned before, LG is an optimal stain in evaluating LWE and Marx line. In TFOS DEWS II Diagnostic Methodology report, repeated instillation of LG by using 2 separate strips wetted with 2 saline drops is recommended for evaluation of LWE, but in a recent study it is found that the number of drops instilled (single versus double) did not significantly affect lid wiper staining, instead repeated lid eversion increased lid wiper staining (Delaveris et al., 2018). For this matter, we performed a single application of LG in each eye, and we ignored eversion of upper eyelid in order to avoid increasing the LWE staining. Furthermore, past studies showed greater staining of the lower lid wiper versus upper lid wiper in dry eye patients who don't use contact lens (Efron et al., 2016). Also, it was difficult to hold patient's upper eyelid behind OCULUS M5 to

record its picture in Covid-19 situation. In lower lid wiper evaluation, we pulled lid down with a cotton soap, without mechanical touching the lid wiper with fingers to avoid impact of fingerprints in LWE. Because lid wiper region is not well defined, it is difficult process for observer to estimate the stained region in comparison to the lid wiper total width and hight. Human observers tend to overestimate the hight and underestimate the width of LWE staining and there are some software to grade the amount of staining (Kunnen, Wolffsohn, & Ritchey, 2018), but in this study we used ready pictures of ULMS eye dryness classification scale by Dr Elena Garcia Rubio to grade LWE in a fast and effective way. By this method we compared taken pictures according to a stable ULMS scale in all cases.

Marx line is a distinct line of cells between the eyelid margin and surface of the bulbar conjunctiva and cornea, and its condition can be used as a simple screening method for assessing meibomian glands function (Yamaguchi et al., 2006). LG is a useful tool to show this line because of the contrast it makes with eye colour structures (Donald, Hamilton, & Doughty, 2003). Similar to the LWE, in this investigation we only observed lower Marx line and coded it as present and absent in participants' eyes.

Statistical results showed a significant difference in Oxford grading scores between the two brands of LG, P- value =2.46e-05 ($P<0.001$). The mean Oxford score for LG1 (**GREEN GLO HUB**) was 0.74 ± 1.41, whereas for LG2 **(I-DEW GREEN**), which is the new brand with CE approval, the mean score of Oxford staining was 0.046 ± 0.26. These findings suggest that GREEN GLO HUB (LG1) might be a better stain for detecting conjunctival and corneal staining than new brand, I-DEW GREEN.
Although, we considered whole three zones as a single Oxford grading for each eye, but since in the recording of the photos by OCULUS-M5 it is challenging to detect corneal staining with LG, the results are more likely related to the conjunctival staining differences rather than corneal staining differences. It is proved that grading of corneal staining based on digital (photographic) images significantly underrepresented the amount of the corneal staining compared to live grading through a slit lamp (Sorbara, Peterson, Schneider, & Woods, 2015), but as said before, this method of grading applied to the both LG brands equally, and it will not affect comparison results.

Five participants (11.11 %) had experience of contact lens (CL) usage, and three of them had their contact lenses when they joined the study on examination days. We recommended to those subjects

to wear CL on the second day of examination same as the first day. They brought the CL out just before applying the LG dyes. As it is becoming apparent that conjunctival abnormalities are associated with dry eye symptoms, researchers recommended LG as an effective stain to evaluate conjunctival disorders, particularly in contact lens wearing patients. It is said that compared to the sodium fluorescein staining, LG staining can discriminate symptomatic and asymptomatic dry eye in contact lens wearer (Guillon & Maissa, 2005). In this study, patients with CL showed half ring-shaped staining around cornea, in the place of the launching CL on conjunctiva. This staining related to the CL was more visible with LG1 and increased LG1 staining score in the study

We used linear regression analysis to identify the strength of the association between the independent variables (age, gender and OSDI score) with Oxford staining score. In this part of statistical analysis, once we tried to see the impact of three variables on whole Oxford staining of LG (Table 3), and after that we examined their effect on the two LG brands separately (Tables 4,5). Based on statistical findings from this study, independent variables like age, OSDI scores and gender didn't have significant effect on Oxford staining scores of two brands of LG (both in general and for each dye separately), and P-value is > 0.05 for all of the measurements. The changes for Oxford scores with increasing 1 scale of age or OSDI score, as continues variables, is not significant. Also, Oxford staining score of LG1 and LG2 will not change significantly with gender (considering male as reference).

Despite of the pervious discussion that states age can influence ocular surface staining (Wang et al., 2020), in this study the association between age and ocular surface staining by LG1 and LG2 was weak, and with 1 unit of increasing age the Oxford score for LG1 and LG2 increased 0.025 and 0.001 respectively (Tables 4,5). Maybe larger sample size is needed to find stronger association between age and LG staining score.

We used Ocular Surface Disease Index (OSDI questionnaire) to evaluate the DED symptoms in subjects and we tried to find relationship between the LG staining scores and OSDI value. Results didn't show statistically significant correlation between OSDI score and Oxford staining by LG1 and LG2. The changes of Oxford scale with increasing 1 unit of OSDI score was minus for both brands of LG, and this shows slight decrease in staining score with increasing OSDI (P>0.05). As reported in many other studies, association between signs and symptoms in dry eye syndrome is low (Bartlett, Keith, Sudharshan, & Snedecor, 2015), also in another study it is proved there is no correlation between disease severity (addressed by the OSDI) and staining patterns by LG and RB(Machado,

Castro, & Fontes, 2009). Therefore, the probable reason that why these two variables (Oxford grading as clinical sign and OSDI score defining symptoms) didn't show regression, could be answered. Also, the mean score of OSDI was 20.19 ±16.7 and this score indicates normal to mild degree of dryness situation in general (Table 3,4,5).

In evaluating gender variable effect on the LG brands Oxford staining, there was not significant association between gender and LG1 and LG2 (p=0.35 for LG1 and p=0.48 for LG2). The slight change in Oxford scores in LG1 was greater for female than male gender (coefficient -0.31 with considering male as reference). And for LG2 the slight change, for male was grater than female gender (coefficient +0.043 with considering male as reference). However, this slight coefficient can not indicate differences between the two brands of LG dyes and their association with gender variable (Table 4,5).

As described in methods, for assessing and grading LWE staining we used reference figures of ULMS eye dryness classification scale by Dr Elena Garcia Rubio (Appendix 2), and LWE is divided into 5 categorical scale, No staining=0 15% staining = 1 25% staining= 2 50% staining=3 100% staining=4. This classification provided us an easy and fast method of grading LWE without measuring width and height of the LWE in subjects.

The current data support that there is a significant difference in staining of LWE between the two LG brands (p=3.827e-06). All tests (100% of 86 samples) came out with category 0 (no staining) LWE for LG2, whereas for LG1 we found 27.89% of cases with grade 1 (15% staining) or above staining (Table1). This result could indicate that LG2 (I-DEW GREEN), is unable in staining LWE and LG1 is better option when it comes to the lid wiper assessment.

Multinomial logistic regression statistical method was used to predict the effect of age, gender and OSDI score on LWE. In this part of analysis, we tested scale 0 (no staining) of LWE as reference to compare the staining correlation with the three independent variables. The only association found in this analysis was relation of age and LWE category 1 (15% staining), with significant p-value (P=0.002). This finding shows that with every 1 unit increase of age (continues variable), the staining of LWE in group (1) will increase 6% in staining, and if we repeat this test many times, in 95% of the tests the increase will happen between 2% and 10% [CI is 1.022-1.1] compared to no staining grade of LWE. Since LG2 didn't present any LWE staining, this correlation is related to the LG1, GREEN GLO HUB (Table6). An earlier survey found that the prevalence of both upper and

lower LWE were significantly correlated with age, but not sex (Shiraishi, Yamaguchi, & Ohashi, 2014), also in another study it is proved that there is a positive association between age and LWE, and LWE is more likely in older patients (Pult, Purslow, & Murphy, 2011). Our recent study improves this findings' integrity when we use LG1 as staining dye, but not LG2.

Results from past studies show that LWE staining is higher in dry eye patients than non-dry eye patients, but no clear relationship and correlation was recognized between LWE and the presence of dry eye (Shiraishi, Yamanishi, Yamamoto, Yamaguchi, & Ohashi, 2009) (Yeniad, Beginoglu, & Bilgin, 2010). Moreover, same studies indicated that more LWEs were detected in contact lens wearers compared to the control groups (Sorbara et al., 2015). OSDI score is defining the severity of the dry eye symptoms, as we can see from results it was not associated with LWE significantly. As discussed earlier based on the studies the association between symptoms and signs of dry eye is low, and on the other hand Shiraishi and colleges proved that there is no marked and clear connection between dry eye and LWE. Having said that, we might conclude why OSDI didn't have significant correlation with LWE in LG staining.

Results show a big difference between two brands of LG in presenting Marx line (P=2.2e-16), similar to the LWE, LG2 didn't show up Marx line in any participants in their right or left eye, and Marx line was notably absent. Instead, LG1 presented Marx line in majority of cases (60.46%). This finding also can prove the failure of LG2 in the clinical usage (Table2).

In logistic regression test for presenting Marx line, with two categories of being present and absent, age and OSDI score didn't show significant relationship with Marx line (P= 372 and P= 326 respectively), but gender variable presented significant p-value (0.025). Interpretation of this relationship with coefficient 2.2 shows that chance of presenting Marx line with LG in males are higher than females. Our finding is inconsistent with other study which indicates that by using LG staining, staining of the Marx line is quantitatively similar in men and women (Doughty, Naase, Donald, Hamilton, & Button, 2004). As Marx line was absent in 100% of examinations with LG2, this significant value in relation with gender is because of the first brand GREEN GLO HUB (LG1).

We didn't find remarkable difference in subjective report of burning sensation between two brands of LG (P= 0.43) (Table 2). Also, age, gender, and OSDI scores didn't show any correlation with the burning sensation scores in subjects totally and separately with each dye (Table 8,9,10). As cited in Methods, in examination forms we described ordinal subjective scales, in which no sensation=0

slight sensation=1 moderate sensation =2 severe sensation=3 described patients' subjective opinion about burning sensation. None of the patients reported severe sensation (grade 3) while applying LG1 and LG2 (Figure1). LG dye changes the Ph of the saline slightly towards acidic direction and this PH could cause discomfort for the patients. In a similar study for comparing performance of different Lissamine green dyes, patients didn't report discomfort with using prepared LG solutions (Delaveris et al., 2018) and since we used LG strips, this idea would be improved that paper strips might cause discomfort while touching with the bulbar conjunctiva, not Lissamine green chemical compound, as the late study claims this discomfort would not be expected to occur with the range of PH measured in the Lissamine green solution (Doughty et al., 2004).

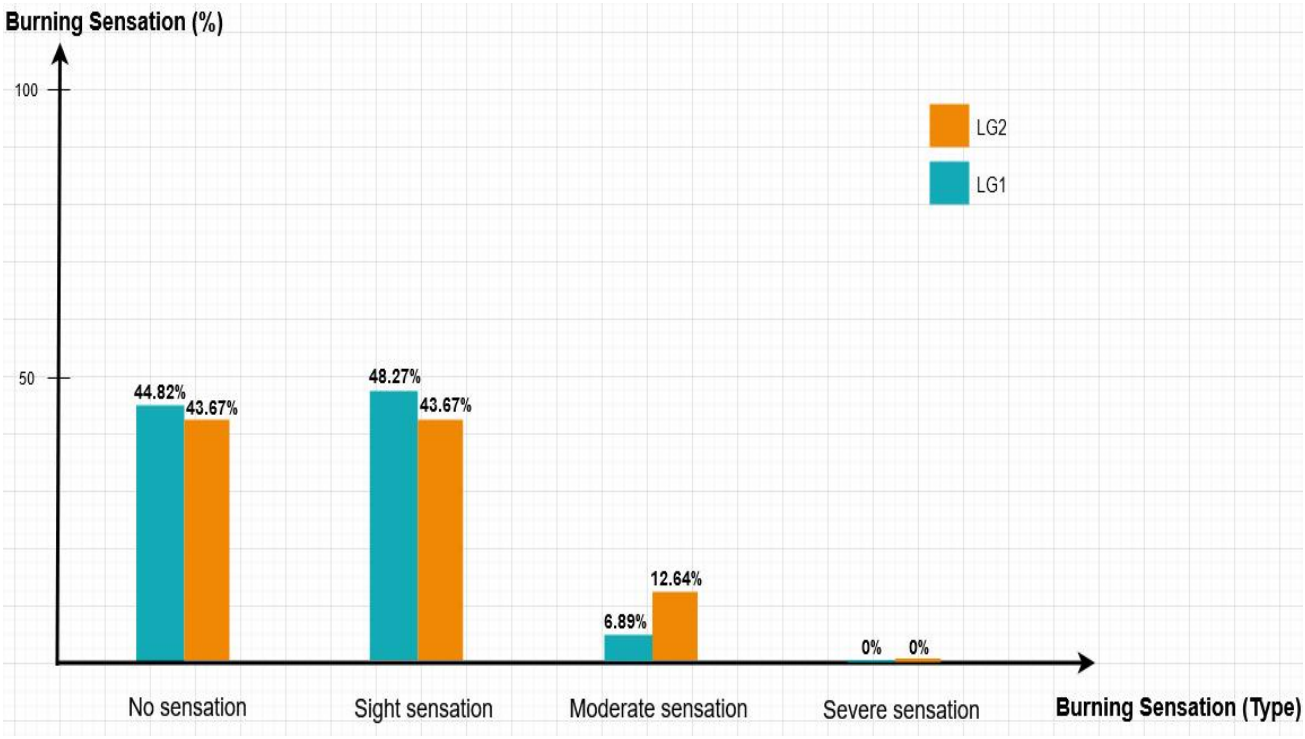

Figure 1: Burning sensation catagorical distribution

Different behavior between two brands of the LG was found while we were using saline to wet the strips. It was said that LG1 retained the saline drop concentrated in the place of drip until shaking the strip and pushing the saline drop to wet whole strip. But in LG2 whole drop go through the strip and wet it immediately after trickle the saline drop and its strip looks thinner compared to the LG1 strip.

Delaveris's research stated that solubility of each dye can differ because of the age of the strips and this solubility could affect the amount of the dye entering to the eye (Delaveris et al., 2018). The only information we had about the age of LG1 and LG2 strips was their expiry date. The expiry date for LG1(GREEN GLO HUB strips) was Sept- 2024, and for LG2 (I-DEW GREEN strips) was JAN – 2022. If we consider similar duration of using time for both strips since manufacturing time, then we can suppose that LG1(GREEN GLO HUB) we used in the study is newer than LG2. And because LG2 is older strip, it has different solubility than LG1 and this affects its performance. In this study, if the strips had same manufacturing time, their solubility behavior and consequently their ability in staining would be more comparable. Furthermore, the thickness of the strips was different and LG1 strips was thicker than LG2. This factor might affect the containing concentration of LG in each strip and might cause stronger performance in LG1.

While taking pictures with OCULUS M5, the place of applying of the dyes had sharper green color while using LG1 and this made a challenging situation in differentiating this area with real conjunctival staining in several cases. Since in all subjects we used the dyes in one specified area in temporal lower conjunctiva, we were cautious about this type of staining when we were grading the staining score based on Oxford scale which is presented by punctate dots.

Figure 2: different behavior of two brands of LG while wetting with saline

Figure 3:different behavior of two brands of LG while wetting with saline

6 Conclusion

Due to the ability of LG in staining of dead structures specifically in ocular structures and non-toxic nature of it, LG has priority to Rose Bengal in recent decades. But not all LG products distinguish questioned structures of eye and different LG products have different capacity of showing damaged eye structures. In recent years, European clinicians started to use I-DEW GREEN LG strips instead of GREEN GLO HUB strips. But the performance of new strips has been questioned. This study was organized to compare the performance of two brands of LG.

From the results of this study we found significant difference between performance of two brands of LG strips in Oxford grading scale for corneal and conjunctival staining (P-value: 2.46e-05) , LWE staining (P-value: 3.827e-06) and Marx line presence (P-value: 2.2e-16). In comparison of burning sensation between two brands of LG the difference was not significant (P-value: 0.43). LG2 didn't show any LWE and Marx line in subjects and the mean of Oxford score for this brand was dramatically lower than LG1. Current study might indicate that LG2 (I-DEW GREEN LG) is an ineffective dye for achieving target goals in ophthalmic examinations.
In investigating the effect of age, gender and OSDI score on two LG performance, we only found significant association of age variable with LWE in category 1 of ULMS grading schema with 15% staining grade (P= 0.002), and significant correlation of gender with Marx line presence (P= 0.025). Since all presence LWE staining and Marx line were related to the LG1, these findings are correlated to this brand of LG (GREEN GLO HUB). OSDI scores didn't show any significant relation with staining scores of LG brands and burning sensation. As mentioned, it might be because of the weak connection of signs and symptoms in situations like dry eye.
To the best of our knowledge, this study is the first study comparing two brands of LG strips and findings supported the different performance of two compared brands. More researches recommended to find out better understanding the effect of age and gender on LG1 performance.

One of the limitations in this study was that we didn't include slit lamp examination to evaluate corneal staining through red filter because of the Covid-19 situation in August 2020. It is suggested that in future studies, researcher use slit lamp and red filter for grading corneal staining by LG dye. Another shortcoming was that we couldn't include more contact lens wearers to find out its effect on two brands of LG in staining LWE. Also, evaluation of upper lid wiper was missing in the study. Furthermore, the study by Delaveris and colleagues discussed about the peak of maximum absorption of light for each brand of dye and how it could impact on LG staining visibility, we didn't

perform this type of analysis. It is said that, some of the components of the LG strips may have limited capacity to bind to the cell structures and washed away easily after a blink (Delaveris et al., 2018). In future studies considering chemical analysis of two brands of I-DEW GREEN LG strips and GREEN GLO HUB strips, might show more results and find the reason of the different performance between these two brands.

Note: The author has no proprietary interest in any of the LG brands used in this study.

ACKNOLEDGMENTS: This study is supported in part by National center of optics, vision and eye care in University of Southeast Norway, Kongsberg.

.

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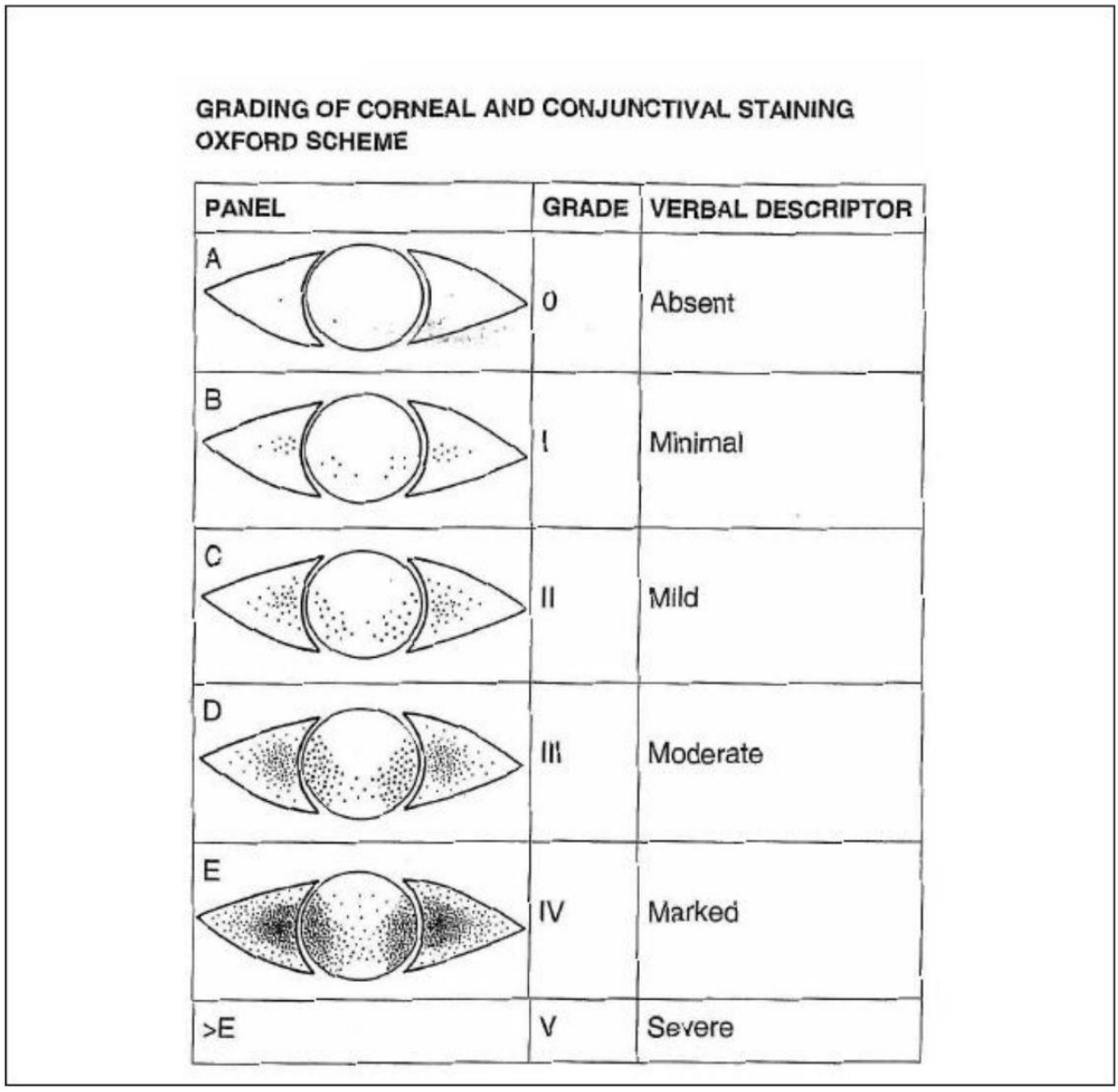
Annexes

Appendix 1: Oxford scheme for grading corneal and conjunctival staining

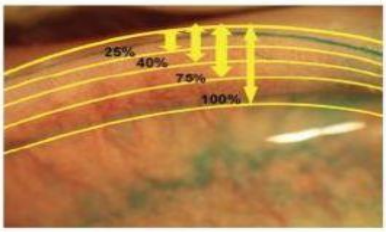


GRADING OF CORNEAL AND CONJUNCTIVAL STAINING
OXFORD SCHEME

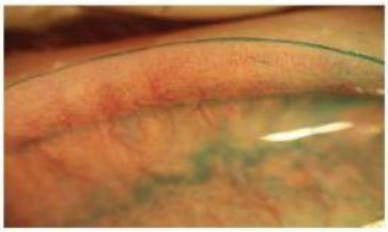
PANEL	GRADE	VERBAL DESCRIPTOR
A	0	Absent
B	I	Minimal
C	II	Mild
D	III	Moderate
E	IV	Marked
>E	V	Severe

Appendix2: Pictures of ULMS eye dryness classification scale by Dr Elena Garcia Rubio

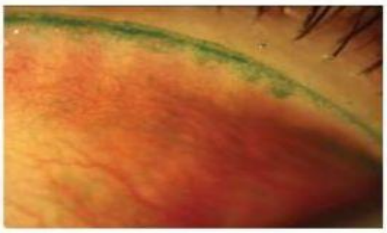
ULMS eye dryness classification scale[2]



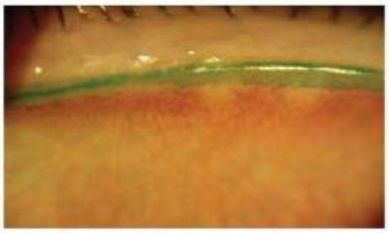
Coverage of upper lid margin staining



No staining



15% staining



25% staining

50% staining

100% staining

All photography by kind permission of Dr Elena Garcia Rubio

Appendix 3: Ocular Surface Disease Index (OSDI questionnaire)

Ocular Surface Disease Index© (OSDI©)[2]

Ask your patient the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

HAVE YOU EXPERIENCED ANY OF THE FOLLOWING *DURING THE LAST WEEK*:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time
1. Eyes that are sensitive to light?	4	3	2	1	0
2. Eyes that feel gritty?	4	3	2	1	0
3. Painful or sore eyes?	4	3	2	1	0
4. Blurred vision?	4	3	2	1	0
5. Poor vision?	4	3	2	1	0

Subtotal score for answers 1 to 5 (A)

HAVE PROBLEMS WITH YOUR EYES LIMITED YOU IN PERFORMING ANY OF THE FOLLOWING *DURING THE LAST WEEK*:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	
6. Reading?	4	3	2	1	0	N/A
7. Driving at night?	4	3	2	1	0	N/A
8. Working with a computer or bank machine (ATM)?	4	3	2	1	0	N/A
9. Watching TV?	4	3	2	1	0	N/A

Subtotal score for answers 6 to 9 (B)

HAVE YOUR EYES FELT UNCOMFORTABLE IN ANY OF THE FOLLOWING SITUATIONS *DURING THE LAST WEEK*:

	All of the time	Most of the time	Half of the time	Some of the time	None of the time	
10. Windy conditions?	4	3	2	1	0	N/A
11. Places or areas with low humidity (very dry)?	4	3	2	1	0	N/A
12. Areas that are air conditioned?	4	3	2	1	0	N/A

Subtotal score for answers 10 to 12 (C)

ADD SUBTOTALS A, B, AND C TO OBTAIN D (D = SUM OF SCORES FOR ALL QUESTIONS ANSWERED) (D)

TOTAL NUMBER OF QUESTIONS ANSWERED (DO NOT INCLUDE QUESTIONS ANSWERED N/A) (E)

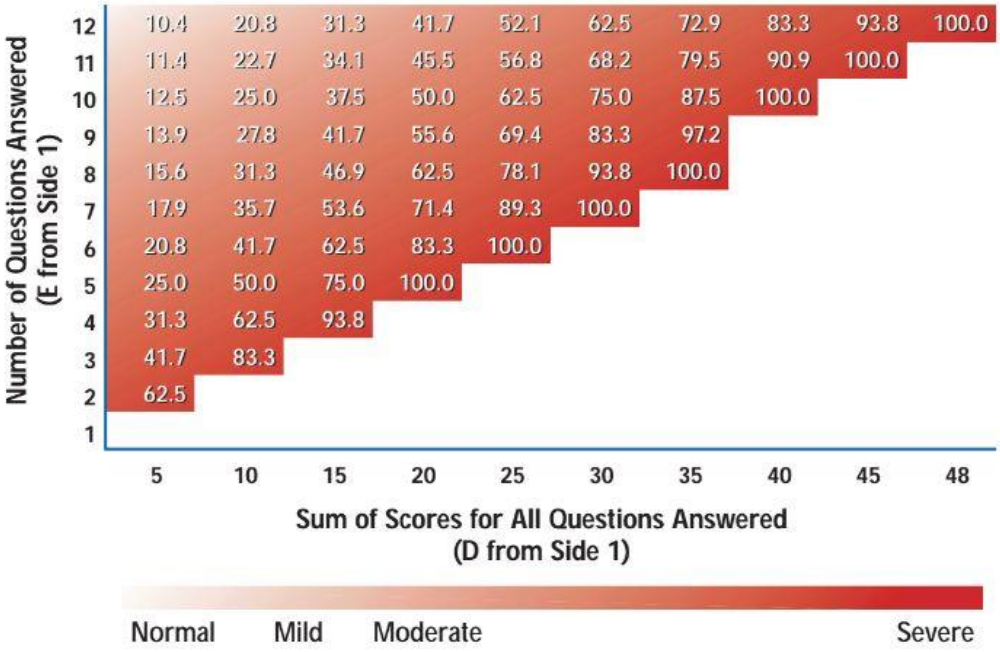
Please turn over the questionnaire to calculate the patient's final OSDI© score.

Evaluating the OSDI© Score[1]

The OSDI© is assessed on a scale of 0 to 100, with higher scores representing greater disability. The index demonstrates sensitivity and specificity in distinguishing between normal subjects and patients with dry eye disease. The OSDI© is a valid and reliable instrument for measuring dry eye disease severity (normal, mild to moderate, and severe) and effect on vision-related function.

Assessing Your Patient's Dry Eye Disease[1,2]

Use your answers D and E from Side 1 to compare the sum of scores for all questions answered (D) and the number of questions answered (E) with the chart below.*
Find where your patient's score would fall. Match the corresponding shade of red to the key below to determine whether your patient's score indicates normal, mild, moderate, or severe dry eye disease.



*Values to determine dry eye disease severity calculated using the OSDI© formula:

$$\text{OSDI©} = \frac{(\text{sum of scores}) \times 25}{(\#\text{ of questions answered})}$$

Patient's Name: _____ **Date:** _____

How long has the patient experienced dry eye? _____

Eye Care Professional's Comments: _____

_____

_____

_____

_____

_____

Restasis® B.I.D. (Cyclosporine Ophthalmic Emulsion) 0.05%

Treat Dry Eye Disease
By Restoring Natural Tear Production

Tear and place in patient's chart for follow-up care on next visit.

Appendix 4: Consent forms by NSD

Vil du delta i forskningsprosjektet

Performance of two different brands of lissamine green strips in staining of ocular surface structures

Dette er et spørsmål til deg om å delta i et forskningsprosjekt hvor formålet er å sammenligne to ulike merker av samme fargestoff som brukes til å se etter tørrhetsskader på øyet i rutineundersøkelser utført av optiker. I dette skrivet gir vi deg informasjon om målene for prosjektet og hva deltakelse vil innebære for deg.

Formål
Lissamine green er et fargestoff som påføres øyet når optiker eller øyelege undersøker om pasienten har tegn på tørre øyne, et vanlig problem i befolkningen (ca 30%). Det finnes flere merker lissamine green på markedet, og det har nettopp kommet et nytt merke som noen optikere har meldt farger dårligere enn andre merker. Det at ulike merker benyttes, kan gjøre at funn, og spesielt endring av funn over tid kan bli oversett av optikeren/øyelegen. Derfor skal dette prosjektet sammenligne i hvilken grad to ulike merker av lissamine green farger øyelokkranden og konjunktiva (det hvite på øyet).

Dette prosjektet er en masteroppgave.

Hvem er ansvarlig for forskningsprosjektet?
Universitetet i Sørøst-Norge (USN) er ansvarlig for prosjektet.

Hvorfor får du spørsmål om å delta?
Denne studien skal undersøke om det er forskjeller mellom to ulike merker fargestoff som bl.a. brukes til å finne ut om overflaten av øynene har tegn på tørrhet. Det stilles ingen spesielle kriterier til personene som ønsker å delta, og informasjon om prosjektet er synliggjort på oppslagstavler på USN Campus Kongsberg. Personer som studerer eller jobber ved USN blir invitert til å delta gjennom direkte forespørsel (muntlig eller e-post). Alle som besøker USN ved Nasjonalt senter for optikk, syn og øyehelse kan be om å få delta.

Hva innebærer det for deg å delta?
I denne studien vil det tas nærbilder i høy forstørrelse av øynene dine på to påfølgende dager til samme tidspunkt. Det vil ikke være mulig å se at bildene er av deg. Før bildene tas, vil øynene dine bli påført et ufarlig fargestoff. Prosedyren er helt smertefri og fargen blir borte av seg selv innen en time.
Anonymiserte bilder av øynene dine (det vil si uten tilknytning til ditt navn eller fødselsdato) lastes ned på en portabel harddisk. En masterstudent vil på et senere tidspunkt gradere i hvor stor grad øyene dine tok opp fargestoffet.
Siden øyne som tar opp mye fargestoff er et tegn på øyetørrhet, vil du i dette prosjektet også bli bedt om å fylle ut et spørreskjema om du har symptomer på tørre øyne. Dette gjøres for å kunne forklare eventuelle forskjeller mellom det to merkene av fargestoff. Dine svar fra spørreskjemaet blir registrert elektronisk, men navnet ditt vil ikke bli registrert. Selve undersøkelsen, inkludert spørreskjema tar ca. 10 minutter per gang.

Det er frivillig å delta
Det er frivillig å delta i prosjektet. Hvis du velger å delta, kan du når som helst trekke ditt samtykke tilbake uten å oppgi noen grunn. Alle opplysninger om deg vil da bli anonymisert. Det vil ikke ha noen negative konsekvenser for deg, ditt forhold til universitetet/underviseren, arbeidsplassen/arbeidsgiveren hvis du ikke vil delta eller senere velger å trekke deg.

Ditt personvern – hvordan vi oppbevarer og bruker dine opplysninger

Vi vil bare bruke opplysningene om deg til formålene vi har fortalt om i dette skrivet. Vi behandler opplysningene konfidensielt og i samsvar med personvernregelverket. Ditt navn og bilder av dine øyne vil lagres på en stasjonær PC på synsklinikken uten tilkobling til nettverk. Når bildene skal analyseres, vil de overføres som rene bildefiler til en portabel PC uten tilknytning til ditt navn eller fødselsdato. Det vil ikke finnes noen liste som knytter ditt navn til dine bilder. Det er kun masterstudenten og veilederne av masterprosjektet som har tilgang til opplysningene om deg.

arbeidsplassen/arbeidsgiveren hvis du ikke vil delta eller senere velger å trekke deg.

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Hva skjer med opplysningene dine når vi avslutter forskningsprosjektet?
Prosjektet skal etter planen avsluttes 20.12.2020. Da vil alle ikke-anonymiserte data som er registrert slettes.

Dine rettigheter
Så lenge du kan identifiseres i datamaterialet, har du rett til:

- innsyn i hvilke personopplysninger som er registrert om deg,
- å få rettet personopplysninger om deg,
- få slettet personopplysninger om deg,
- få utlevert en kopi av dine personopplysninger (dataportabilitet), og
- å sende klage til personvernombudet eller Datatilsynet om behandlingen av dine personopplysninger.

Hva gir oss rett til å behandle personopplysninger om deg?
Vi behandler opplysninger om deg basert på ditt samtykke.

På oppdrag fra Universitetet i Sørøst-Norge har NSD – Norsk senter for forskningsdata AS vurdert at behandlingen av personopplysninger i dette prosjektet er i samsvar med personvernregelverket.

Hvor kan jeg finne ut mer?
Hvis du har spørsmål til studien, eller ønsker å benytte deg av dine rettigheter, ta kontakt med:

- *[Universitetet i Sørøst-Norge]* ved *[veileder og prosjektansvarlig Bente Monica Aakre, på epost (Bente.M.Aakre@usn.no) eller telefon: 913 58 525.]. I studentprosjekt må kontaktopplysninger til veileder/prosjektansvarlig fremgå, ikke kun student*
- Vårt personvernombud: Paal Are Solberg, på e-post (Paal.A.Solberg@usn.no) eller telefon: 918 60 041.
- NSD – Norsk senter for forskningsdata AS, på epost (personverntjenester@nsd.no) eller telefon: 55 58 21 17.

Med vennlig hilsen

Bente Monica Aakre
Prosjektansvarlig
(Forsker/veileder)

Eventuelt student

Samtykkeerklæring

(Forsker/veileder)

Samtykkeerklæring

Jeg har mottatt og forstått informasjon om prosjektet *Performance of two different brands of lissamine green strips in staining of ocular surface structures*, og har fått anledning til å stille spørsmål. Jeg samtykker til:

- ☐ at det blir tatt bilder av øynene mine, to påfølgende dager etter påføring av fargestoffet Lissamine Green*[sett inn aktuell metode, f.eks. intervju]*
- ☐ å fylle ut et spørreskjema om symptomer på tørre øyne

Jeg samtykker til at mine opplysninger behandles frem til prosjektet er avsluttet, ca. 20.12.2020

(Signert av prosjektdeltaker, dato)

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