lipid layer of the tear film to be approximately 40 nm [395]. Since this initial analysis, single-wavelength interferometry has been applied to such measurements [396–399]. Guillon et al. developed a clinical interferometer (Tearscope; Keeler, Windsor, UK) that uses broadband illumination to visualize the kinetics of the lipid layer of the tear film, showing that different patterns of interferometric fringe are generated according to the lipid layer thickness [154]. Goto et al. developed an algorithm for quantifying lipid layer thickness from interferometric fringe patterns [398]. The DR-1 system (Kowa, Nagoya, Japan) was also developed as an interferometer for evaluation of the kinetics of the lipid layer of the tear film in both normal subjects and patients with DED (Fig. 3). This system has revealed that lipid layer kinetics are related to the tear film condition or blink pattern [398]. Interferometry is now an established technique for clinical examination that allows visualization of the kinetics of the oily layer of the tear film.

The LipiView interferometer (TearScience, Morrisville, NC) was recently introduced as the first clinically available instrument to allow automated measurement of the thickness of the lipid layer of the tear film [153] This instrument has a sensitivity of 65.8% and a specificity of 63.4% using a cut-off value of 75-nm for the detection of MGD, but its diagnostic contribution to DED has not been established [153]. The lateral shearing interferometer has also recently been introduced for research purposes [400–403]. This latter system relies on illumination with a helium-neon laser, and analysis by fast Fourier transform, to evaluate surface irregularities of the tear film related to breakup of the lipid layer. Such instruments are likely to provide new insights into the lipid layer of the tear film and the pathophysiology of dry eye.

6.8.1.2.3. Meibography. Meibography allows observation of the silhouette of the meibomian gland morphological structure. The original technique involved white-light transillumination of everted eyelids from the skin aspect, with imaging based on black-and-white film [404], infrared film [405–407], and a near-infrared charge-coupled device (CCD) video camera [408]. Arita et al. developed a non-contact, slit lamp mounted meibography system that relies on an infrared filter and an infrared CCD video camera, in which imaging is less time-consuming than other systems (Fig. 4) [409]. Recent advances in technology have led to the development of several mobile, handheld, pen-shaped and multi-functionality systems with infrared light-emitting diodes (LEDs) fixed to infrared cameras that allow the capture of videos and images of similar quality to those obtained with earlier meibography systems [410–412].

Several different scoring scales, such as the meiboscore, have been proposed for the evaluation of meibography [409,411,413–416]. In addition, quantitative evaluation of meibomian gland area visualized by meibography has been performed [417–420]. Such quantitative evaluation has been applied to the diagnosis of MGD [419] as well as to evaluation of the effects of treatment [421,422]. Meibography alone does not appear to be sufficient for the diagnosis of MGD, but instead should be interpreted in the context of other clinical parameters [411,423–425]. The thickness of the lipid layer of the tear film measured by interferometry (LipiView) was found to be related to meibomian gland area determined by meibography [426]. Tear fluid secretion has also been shown to be positively correlated, as a compensatory mechanism, with the area devoid of meibomian glands in patients with MGD [427].

Diagnostic cut-off values for the meiboscore in combination with symptoms and lid margin abnormalities demonstrated a sensitivity of 84.9% and specificity of 96.7% for the diagnosis of MGD, in a study comparing normal eyes with those affected by obstructive MGD [423]. Meibography scales have been found to be highly reproducible [413,428], Meibography has revealed that changes in meibomian gland morphology are less pronounced in patients with ADDE than EDE [427,429]. However, shortening of meibomian gland ducts was frequently detected in wearers of contact lenses who complained of DED symptoms [430]. Establishing the diagnostic value of meibography in DED requires further study.

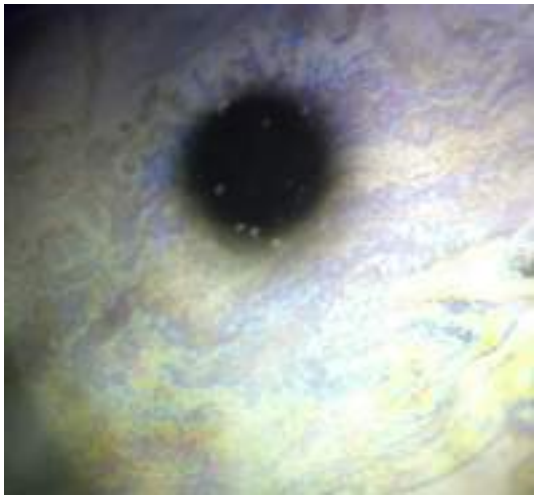


Fig. 3. Interferometric image of the tear film lipid layer in a patient with dry eye. An abnormal multicolored interferometric fringe is observed. A video of lipid layer imaging is also available on the TFOS website.

Table 5
LWE grading scale [391].

Horizontal length of staining	Grade	Sagittal width of staining	Grade
<2 mm	0	<25% of the lid wiper	0
2-4 mm	1	25% - <50% of the lid wiper	1
5-9 mm	2	50% - <75% of the lid wiper	2
>10 mm	3	≥75% of the lid wiper	3

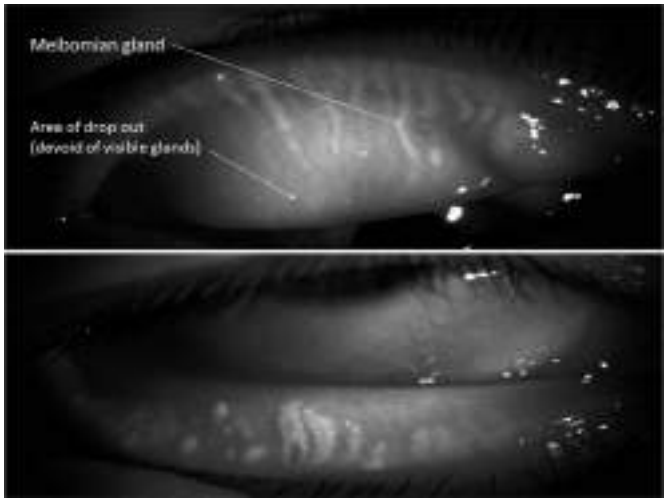


Fig. 4. Infrared images of the upper and lower eyelids obtained by non-invasive meibography in a patient with MGD. Hyper-illuminated regions correspond to meibomian glands. Note that dark areas of gland dropout, presumed to indicate gland atrophy, as well as gland shortening, are apparent.

6.8.1.2.4. Meibomian gland expressibility/duct assessment. Meibomian glands secrete meibum, which contains components of the lipid layer of the tear film. Meibum quantity, quality and expressibility are thought to reflect meibomian gland function. The expressibility of meibum, as an indicator of meibum secretion, is commonly determined by the application of digital pressure to the glands, along the length of the eyelid, through the skin surface of the eyelid [406,431,432], although more standardized procedures for expression have been reported [433]. In the normal eyelid, meibum is clear and readily expressed with gentle pressure. Conversely, the condition of meibum in patients with MGD is varied. In such individuals, meibum can lose its clarity to become cloudy and then opaque and its viscosity can be increased, becoming toothpaste-like and difficult to express in patients with severe MGD. The ranging qualities of meibum as well as its expressibility have been evaluated in various grading schemes. The number and location of expressible glands, as well the response of the glands to different levels of digitally applied pressure, have thus been scored and graded, providing information directly related to meibomian gland condition [416,433–439]. However, the diagnostic value of meibomian gland expressibility and duct appearance has not been established in DED.

6.8.1.3. In vivo confocal imaging. IVCM can be used to study the eyelid margin, to diagnose eyelid mite infestation [440,441], and to assess meibomian gland changes [329,442]. This technology has shown diagnostic benefits in obstructive MGD, providing new information about meibomian gland morphology related to specific conditions, such as contact lens wear, GVHD and atopic keratoconjunctivitis [368,443–447], and could detect the response of meibomian glands to treatment [448,449].

6.8.1.4. Dynamic

6.8.1.4.1. Blink/lid closure analysis. Blinking is vital in maintaining optical performance and the health of the ocular surface. The blinking action clears debris, provides mechanical protection and re-forms the tear film [107,393,450–459]. Furthermore, blinking appears to be vital for meibum distribution [460], and in re-forming a proper tear film lipid layer [450,451,454]. The percentage of almost complete blinks is correlated to DED symptoms and LIPCOF, perhaps due to physical interference with spontaneous blinks [298,301], and may be related to MG morphology [461]. However, there is a broad spectrum of reported results, between 10% and 80%, for the percentage of incomplete blinks in a population of healthy individuals [454,455,462–464]. This may be due to the different measurement protocols and procedures, or variations in the visual task, or the eyelid motion detection method.

The normal spontaneous blink rate is reported to occur from 10 to 15 blinks per minute [301,465–467]. It is higher in females than in males [301,463,464], but the effect of age is controversial [301,467,468]. Incomplete blinking can result in DED and exposure keratopathy [301,452,469]. The inter-blink interval is variable between subjects, is decreased in DED and can be increased with artificial tear instillation [301,452,470]. However, the blink rate is also affected by systemic conditions such as Parkinson disease [471], and tasks such as computer work [472].

Blink speed is faster in the closing phase than the opening phase and faster for the upper lid than for the lower lid [473]. There appear to be no correlation between blink speed and either DED symptoms or tear film stability. However the upper lid velocity is positively related to LIPCOF [295,473].

Incomplete blinks can result in DED symptoms and corneal staining observable by slit lamp biomicroscope. Using fluorescein, the incomplete blink can be highlighted by a “tide line” visible as a dark line in the fluorescein pattern indicating the lower limit of movement of the upper eyelid during a recent incomplete blink [464]. More advanced methods utilise high speed video, possible now even on smart phones [474], observed from an inferior-temporal angle [301,475] However appropriate diagnostic cut-off values and sensitivity and specificity figures still require investigation.

6.8.1.4.2. Lid sensitivity. Ocular surface sensitivity plays a role in the maintenance of ocular surface homeostasis. A Cochet-Bonnet esthesiometer has been applied to evaluate lid sensitivity in several studies. Norn found that lid sensitivity was intermediate between corneal sensitivity and conjunctival sensitivity in healthy subjects [476,477], and others have reported the lower eyelid is more sensitive than the upper eyelid [478,479]. Lid margin sensitivity was found to be normal in patients with chronic blepharitis or DED [477]. It thus remains unclear whether lid sensitivity may show disease-dependent changes or whether it is unaffected in eyelid disease.

6.8.2. Diagnostic test recommendation and technique

For subtype classifying of DED and to inform appropriate management, the presence of blepharitis, and their blink rate and completeness when a patient is performing a task such as completing a DED questionnaire, unaware that the eye care practitioner is observing them, should be noted. Lipid thickness should be observed with an interferometric technique and the pattern graded. Ideally meibography should be performed along with duct observation and expressibility [480].

7. Monitoring dry eye disease progression and management

Few studies have monitored changes in DED signs and symptoms over time. New electronic technologies, such as smartphones or other handheld devices, have been tested recently to capture symptom information in “real time” rather than rely on reports from a recall period, thus aiding patient monitoring [481].

The Women's Health Study and Physicians' Health Study cohorts, revealed worsening of vision-related symptoms in 29% of the subjects since diagnosis (on average 10.5 years). In multivariable logistic regression models for visual symptoms, spending >$20 (USD) per month on DED treatments, presence of a history of severe DED symptoms, and use of systemic beta-blockers were significantly associated with patient-reported visual worsening. Patients who reported severe symptoms of DED in the past were more likely to report worsening and to have corneal staining, suggesting that this might be a clinically relevant indicator of the probability of visual/OSD progression [482]. More prospective studies monitoring visual changes during the natural course of DED, and following treatment, are needed in the future.

8. Clinical protocol for dry eye diagnostic test battery

From Section 6, the recommended diagnostic and monitoring test battery is collated in Fig. 5. Symptoms and at least one positive result of the markers of homeostasis listed below should constitute the diagnosis of DED. If a patient has dry eye symptoms, DED is diagnosed when at least one homeostasis test result is positive. This can occur even if the practitioner does not have access to the full battery of recommended tests. However, if the practitioner has access to only a limited number of the homestasis marker tests and these show negative results, a referral may be necessary to confirm the results of the remaining measures, to which the practitioner does not have access, before a diagnosis of DED can be excluded.

In situations where there are chronic symptoms but limited signs, that are refractory to treatment, then neuropathic pain rather than DED should be considered. Asymptomatic patients with DED

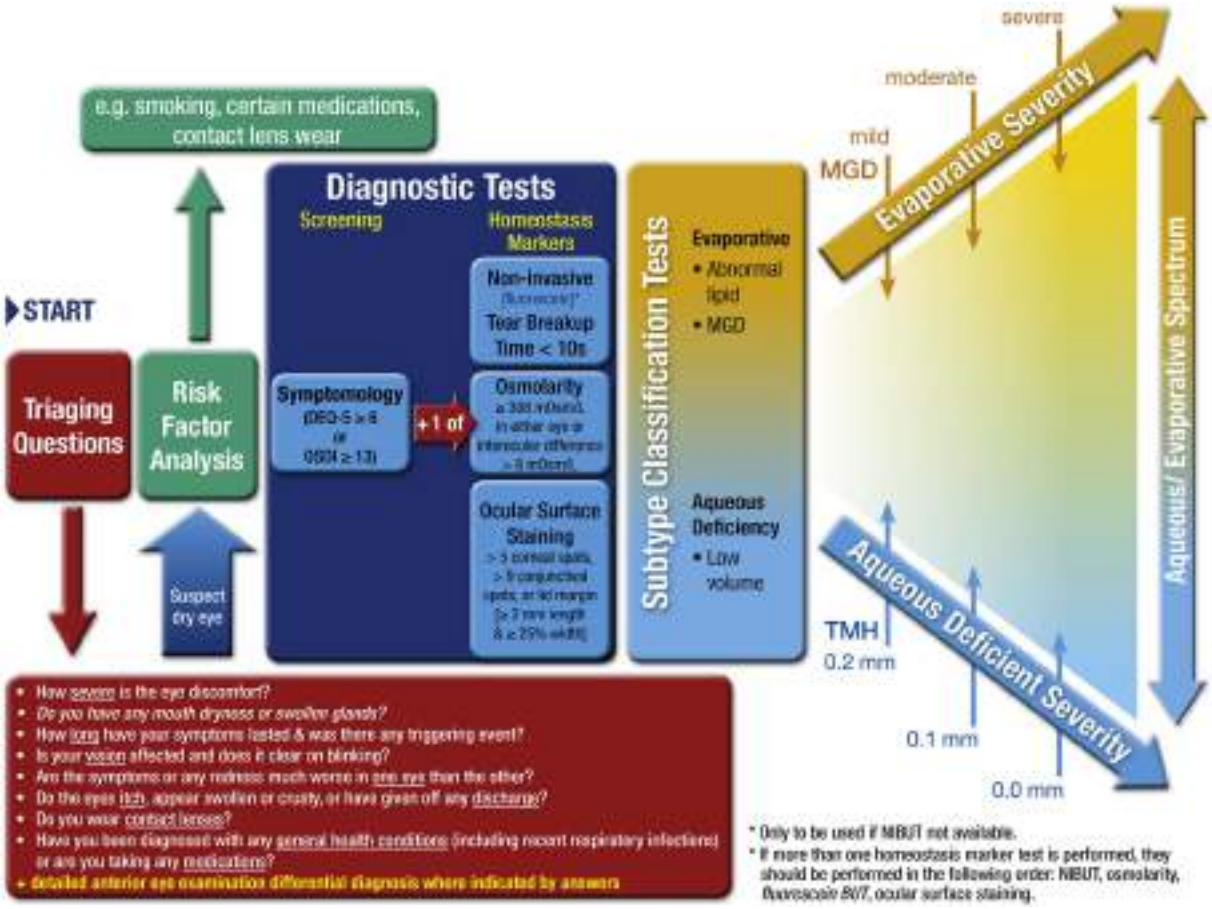


Fig. 5. DED diagnostic test battery. The screening DEQ-5 or OSDI confirms that a patient might have DED and triggers diagnostic testing of non-invasive breakup time, osmolarity [measured prior to breakup time if FBUT used] and ocular surface staining with fluorescein and lissamine green (observing the cornea, conjunctiva and eyelid margin). On initial diagnosis, it is important to exclude conditions that can mimic DED with the aid of the triaging questions (Section 9) and to assess the risk factors which may inform management options [380]. Marked symptoms in the absence of clinically observable signs might suggest an element of neuropathic pain. DED is a subset of OSD; signs alone may still warrant management to prevent DED manifestation and to optimise the optical corneal surface such as prior to refractive surgery or contact lens wear [4]. MGD features [483], lipid thickness/dynamics, and tear volume assessment, and their severity inform the subtype classification of DED as predominantly evaporative or predominantly aqueous deficient which helps inform the management of DED. In accordance with the recommendations of the MGD Workshop (2011) [483], MILD MGD is indicated by a secretion grade 4-7, an expressibility grade of 1 and an amorphous/color fringe lipid pattern. MODERATE MGD is indicated by meibomian gland orifice plugging, lid margin vascularity, a secretion grade 8-12, an expressibility grade of 2 and a meshwork or wave (flow) lipid pattern. SEVERE MGD is indicated by lid margin meibomian gland orifice drop-out or displacement, a secretion grade $\geq$ 13, an expressibility grade of 3 and an absent, globular or abnormal color fringe lipid pattern. Videos of these diagnostic and sub-classification techniques are available on the TFOS website. Sjögren syndrome should be suspected if the DEQ-5 score is > 12. Further testing will help identify treatment mechanisms worthy of targeting, but are beyond the scope of this Diagnostic Methodology report.

type signs, unattributable to other conditions via the differential diagnosis and comorbidities triaging questions in Section 9, might still warrant prophylactic ocular surface treatment. Videos of these diagnostic as well as sub-classification techniques of MGD, lipid thickness/dynamics and tear volume are available on the TFOS website (https://www.theocularsurfacejournal.com).

Tables of severity describing several signs and symptoms and (often arbitrary) cut-offs for different levels are of limited use, as features of dry eye often do not show strong association. Hence it is recommended that severity, for the purpose of selecting treatment, is based on subtype classification features (MGD, lipid thickness/dynamics and non-invasive tear volume) along with symptomology.

The recommended order and clinical practice procedural recommendations are as follows:

8.1. Symptoms

DEQ-5 (Fig. 6a) or OSDI (Fig. 6b) – self-administered [35,37]. Positive result is a DEQ-5 score $\geq$6 [37], or OSDI score $\geq$13 [35].

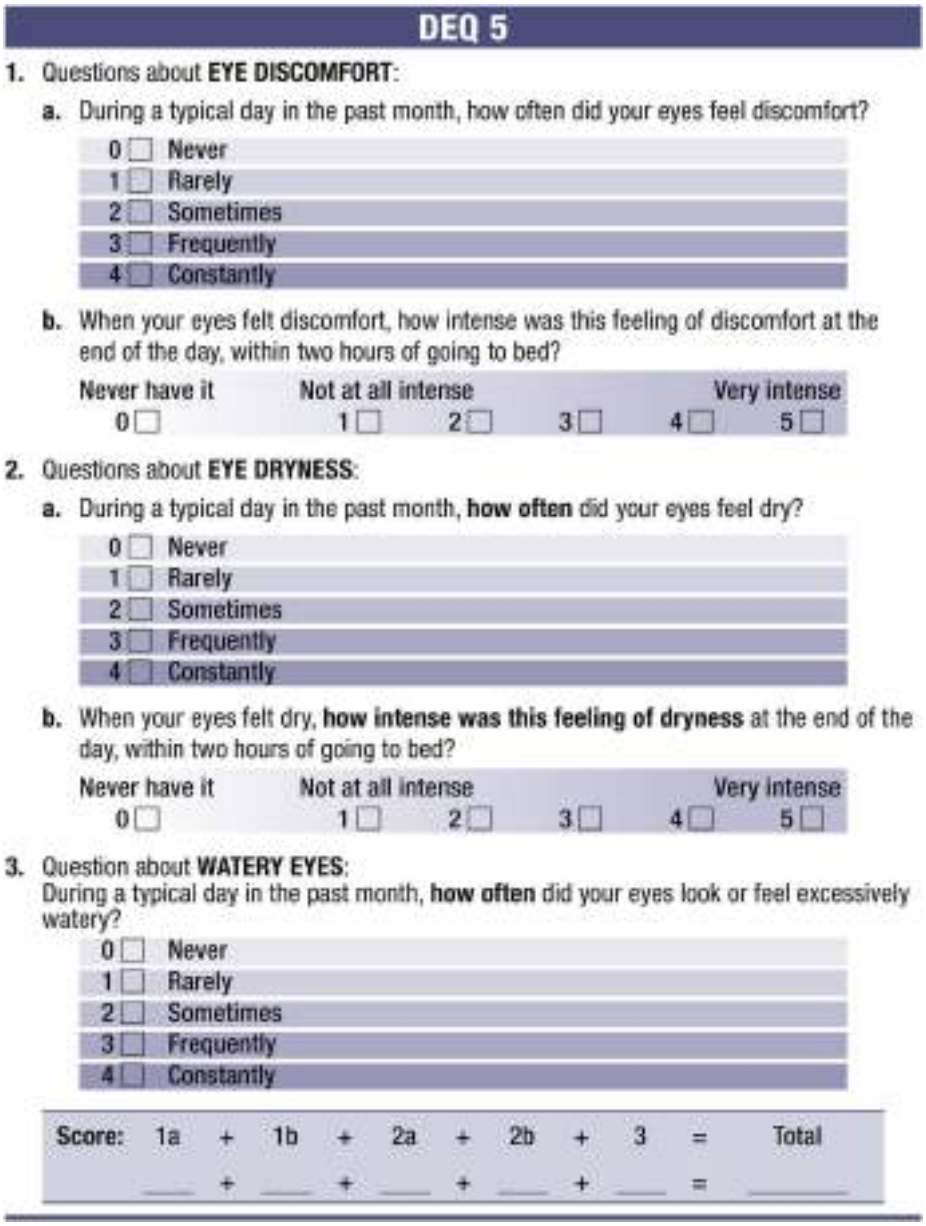
8.2. Tear breakup time

8.2.1. Non-invasive breakup time

Non-invasive breakup time should be performed with a method where as much of the naturally exposed cornea as possible is specularly illuminated with a light source allowing observation of breakup over the whole surface, after a blink. Objective methods are preferred, with three measurements being performed and the median value recorded. Following training, if a patient can no longer refrain from blinking before the tear film breaks up, this is typically counted as the breakup time for that measurement [194]. The lower median breakup value of the two eyes should be considered in making the diagnosis. The cut-off for a positive finding can be as low as 2.7 s for automated algorithms [142], and up to 10 s for subjective observation techniques [134].

8.2.2. FBUT

FBUT can be considered when non-invasive techniques are not available, but due to its more invasive nature, should follow after osmolarity measurement. Fluorescein should be instilled at the



DEQ 5

1. Questions about **EYE DISCOMFORT**:
   a. During a typical day in the past month, how often did your eyes feel discomfort?
   - 0 ☐ Never
   - 1 ☐ Rarely
   - 2 ☐ Sometimes
   - 3 ☐ Frequently
   - 4 ☐ Constantly

   b. When your eyes felt discomfort, how intense was this feeling of discomfort at the end of the day, within two hours of going to bed?

Never have it	Not at all intense				Very intense
0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

2. Questions about **EYE DRYNESS**:
   a. During a typical day in the past month, **how often** did your eyes feel dry?
   - 0 ☐ Never
   - 1 ☐ Rarely
   - 2 ☐ Sometimes
   - 3 ☐ Frequently
   - 4 ☐ Constantly

   b. When your eyes felt dry, **how intense was this feeling of dryness** at the end of the day, within two hours of going to bed?

Never have it	Not at all intense				Very intense
0 ☐	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

3. Question about **WATERY EYES**:
   During a typical day in the past month, **how often** did your eyes look or feel excessively watery?
   - 0 ☐ Never
   - 1 ☐ Rarely
   - 2 ☐ Sometimes
   - 3 ☐ Frequently
   - 4 ☐ Constantly

Score:	1a	+	1b	+	2a	+	2b	+	3	=	Total
	____	+	____	+	____	+	____	+	____	=	_____

Fig. 6a. Five-item Dry Eye Questionnaire (DEQ-5) reproduced with permission (Indiana University) [37].

outer canthus to avoid ocular surface damage (see Section 8.4.1 for instillation instructions), with the excess saline on the strip shaken off, or a reduced area fluorescein strip used [118]. For optimal results, viewing should take place between 1 and 3 min after instillation [279]. A positive finding has been reported to be a value < 10 s [13] although in some studies the average in healthy middle aged patients is noted to be lower than this [244].

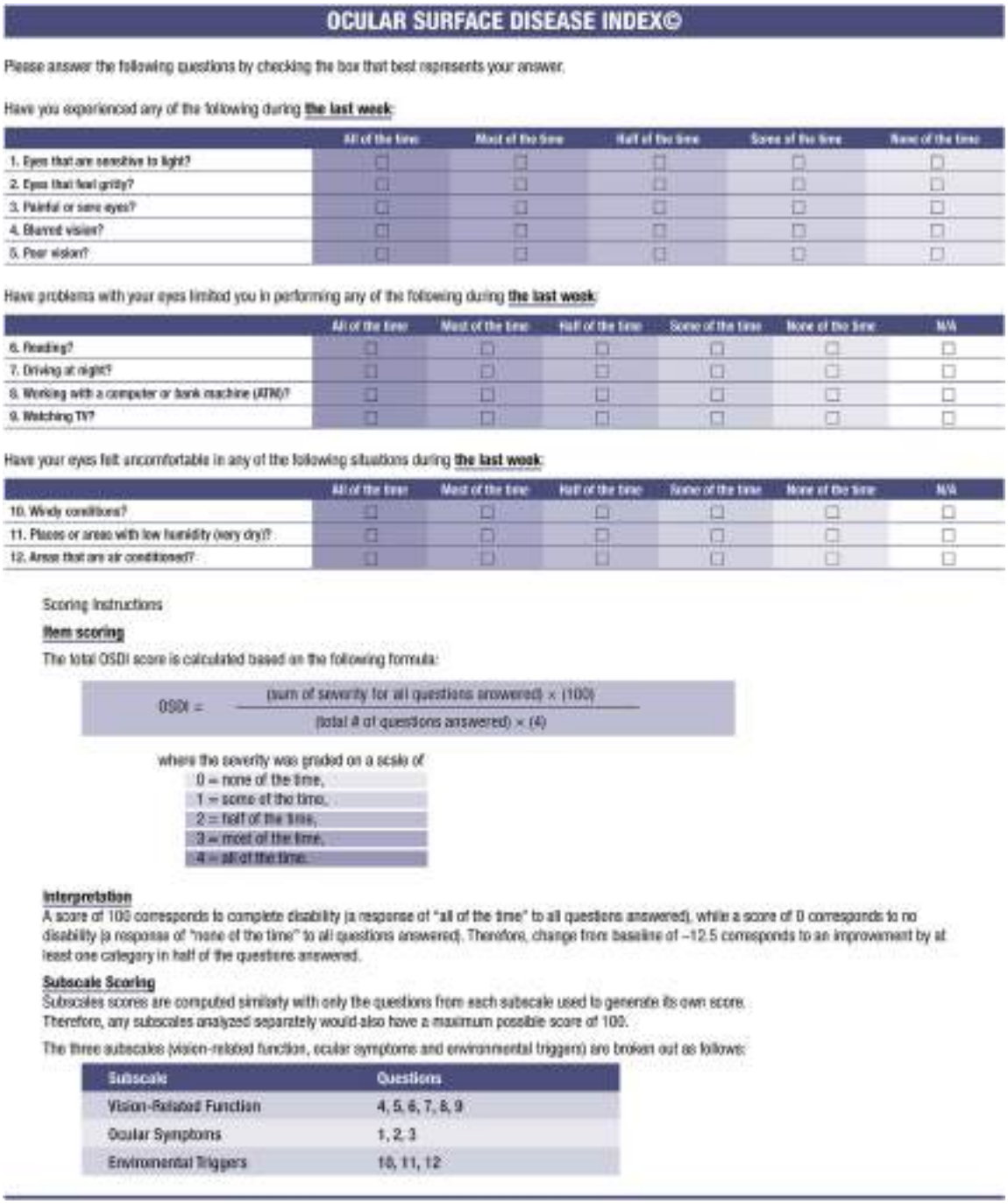
8.3. Osmolarity

Osmolarity should be assessed with a temperature stabilised, calibration checked device. In the case of the Tearlab, temperature stability is achieved by having the device powered on for a sufficient period of time with test cards adjacent to the device for at least 30 min. Seat the patient with chin tilted upward and eyes directed toward the ceiling. Place one hand on the face for stabilization, as appropriate. Do not pull the eyelid down or away from the eye. Sample from just above the lower eyelid tear meniscus, being careful not to press inward to avoid contact with the globe during collection. The difference between the eyes as well as the absolute measures can be diagnostic [170,171]. A positive result is considered to be ≥ 308 mOsm/L with the currently available device in either eye [13,15], or an interocular difference >8 mOsm/L [171].

8.4. Ocular surface staining

8.4.1. Lissamine green staining

Principally for assessing conjunctival and lid margin damage, a lissamine green strip is wet with saline, with the whole drop retained on the strip for at least 5 s to elute the dye. A 10 μL or ~ ¼ to ½ of a drop appears to be an optimal volume if pipetting a



OCULAR SURFACE DISEASE INDEX©

Please answer the following questions by checking the box that best represents your answer.

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?	☐	☐	☐	☐	☐
2. Eyes that feel gritty?	☐	☐	☐	☐	☐
3. Painful or sore eyes?	☐	☐	☐	☐	☐
4. Blurred vision?	☐	☐	☐	☐	☐
5. Poor vision?	☐	☐	☐	☐	☐

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	N/A
6. Reading?	☐	☐	☐	☐	☐	☐
7. Driving at night?	☐	☐	☐	☐	☐	☐
8. Working with a computer or bank machine (ATM)?	☐	☐	☐	☐	☐	☐
9. Watching TV?	☐	☐	☐	☐	☐	☐

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	N/A
10. Windy conditions?	☐	☐	☐	☐	☐	☐
11. Places or areas with low humidity (very dry)?	☐	☐	☐	☐	☐	☐
12. Areas that are air conditioned?	☐	☐	☐	☐	☐	☐

Scoring Instructions

Item scoring

The total OSDI score is calculated based on the following formula:

$$OSDI = \frac{(\text{sum of severity for all questions answered}) \times (100)}{(\text{total \# of questions answered}) \times (4)}$$

where the severity was graded on a scale of

0 = none of the time,
1 = some of the time,
2 = half of the time,
3 = most of the time,
4 = all of the time.

Interpretation

A score of 100 corresponds to complete disability (a response of "all of the time" to all questions answered), while a score of 0 corresponds to no disability (a response of "none of the time" to all questions answered). Therefore, change from baseline of −12.5 corresponds to an improvement by at least one category in half of the questions answered.

Subscale Scoring

Subscales scores are computed similarly with only the questions from each subscale used to generate its own score. Therefore, any subscales analyzed separately would also have a maximum possible score of 100.

The three subscales (vision-related function, ocular symptoms and environmental triggers) are broken out as follows:

Subscale	Questions
Vision-Related Function	4, 5, 6, 7, 8, 9
Ocular Symptoms	1, 2, 3
Environmental Triggers	10, 11, 12

Fig. 6b. Ocular Surface Disease Index (OSDI©) Version 1 Permission received. Copyright 1995 Allergan Inc. Irvine, CA, USA. All rights reserved.

predetermined concentration solution [272,280]. Otherwise, a drop from the strip is instilled inside the far lower temporal lid in upgaze with the lower eyelid of the eye pulled slightly temporally to avoid damage to the conjunctival or lid wiper tissue (Fig. 7). Studies have suggested that observation should occur between 1 and 4 min post-instillation, and that observation through a red filter potentially aids visualization [272,280]. A positive score is > 9 conjunctival spots [285].

8.4.2. Fluorescein staining

Principally for assessing corneal damage, fluorescein should be instilled in a similar way, but with the excess saline on the strip shaken off to instil a minimal volume. Optimal viewing is between 1 and 3 min after instillation [279]. A positive result is > 5 corneal spots [285].

Lid wiper epitheliopathy can be observed stained with fluorescein, rose bengal or lissamine green dyes, although there seems to be a preference for just lissamine green in recent research, with viewing recommended 3–6 min after repeat instillation using 2 separate strips wet with 2 saline drops [484]. Positive is LWE of ≥ 2 mm in length and/or ≥ 25% sagittal width (excluding Marx's line) [391].

Fig. 7. Recommended location to apply ophthalmic dyes in strip form to avoid confounding damage to the conjunctiva and lid margins observed for the diagnosis of DED and its sub-classification. See video on TFOS website for further guidance.

DED severity can change with the time of day so this should be considered in interpreting results and in monitoring DED over time [485,486].

9. Differential diagnosis & comorbidities

Based on the conditions that can mimic the signs and symptoms of DED outlined in the subsections below, administering a series of questions (Table 6) will aid in the differential diagnosis. While further investigation of possible comorbidities should not negate immediate relief management of DED-type symptoms, failing to fully investigate possible comorbidities can lead to non-optimized treatment and the delayed diagnosis of causative conditions that could have serious consequences, such as the higher risk of malignancy in Sjögren syndrome [487]. If questioning by non-eye care professionals suggests DED, but recommended treatments do not result in a marked improvement in symptoms within about a one-month period, a detailed eye examination is recommended.

For those patients where the history-taking for differential diagnosis suggests that this might not be primary DED, a full differential diagnosis should be performed using a slit lamp biomicroscope to examine the:

- eyelashes for both anterior blepharitis and signs of demodex infestation
- eyelid palpebral conjunctiva for MGD and the presence of follicles or swelling
- bulbar conjunctiva for redness pattern and signs of swelling
- cornea for ulceration, and staining should be applied to detect possible trauma
- anterior chamber for the presence of cells or flare, indicating intraocular inflammation

9.1. Conjunctivitis

9.1.1. Allergic conjunctivitis

Symptoms of DED may be very similar to those of allergic conjunctivitis and the conditions can occur simultaneously [380]. In one study of 689 patients with Sjogren Syndrome, clinically

Table 6
Initial questions for the differential diagnosis of DED, indicating where more detailed observation of the ocular surface and adnexa is warranted. Medications which can cause DED are noted in the TFOS DEWS II Epidemiology report [57]. Sjögren syndrome is a subtype of DED, but is included in the differential diagnosis questioning to ensure it is considered from the outset.

How severe is the eye discomfort?	•Unless severe, dry eye presents with signs of irritation such as dryness and grittiness rather than 'pain'. If pain is present, investigate for signs of trauma / infection /ulceration.
Do you have any mouth dryness or enlarged glands?	•*Trigger for Sjogren syndrome investigation*
How long have your symptoms lasted & was there any triggering event?	•Dry eye is a chronic condition, present from morning to evening but generally worse at the end of the day, so if sudden onset or linked with an event, examine for trauma / infection / ulceration.
Is your vision affected and does it clear on blinking?	•Vision is generally impaired with prolonged staring, but should largely recover after a blink; a reduction in vision which does not improve with blinking, particularly with sudden onset, requires an urgent ophthalmic examination.
Are the symptoms or any redness much worse in one eye than the other?	•Dry eye is generally a bilateral condition, so if symptoms or redness are much greater in one eye than the other, detailed eye examination is required to exclude trauma & infection
Do the eyes itch, are they swollen, crusty or have they given off any discharge?	•Itching is usually associated with allergies while a mucopurulent discharge is associated with ocular infection
Do you wear contact lenses?	•Contact lenses can induce dry eye signs and symptoms and appropriate management strategies should be employed by the contact lens prescriber.
Have you been diagnosed with any general health conditions (including recent respiratory infections) or are you taking any medications?	•Patients should be advised to mention their symptoms to the health professionals managing their condition, as modified treatment may minimise or alleviate their dry eye.

significant itching was found in 194 (28.2%) cases; DED was reported to be a symptom in 247 (35.8%) cases; and redness was documented in 194 (28.2%) cases [487]. Systemically, the presence of immunoglobulin E (IgE) antibodies to seasonal or perennial allergens can be documented in most cases of allergic conjunctivitis [488], and there are now some diagnostic tests available to indicate the presence of IgE biomarkers in the tear film or on the ocular surface. In addition, classical allergic conjunctivitis clinical findings, such as conjunctival chemosis, eyelid edema and conjunctival papillae, differentiate allergic from DED [489,490]. Also, allergic rhinitis is present in more than 80% of ocular allergy cases [491,492], but is not a symptom known to be associated with DED. Other findings frequently detected in allergy include a strong family history, atopic dermatitis and/or the presence of asthma [493]. Common oral pharmaceutical agents for allergy treatment have a significant drying effect on the ocular surface and may actually induce DED in patients [494,495]. A diminished tear volume, in turn, permits allergens to remain on the surface longer and may induce or exacerbate allergic conjunctivitis [496].

Giant papillary conjunctivitis (GPC) is associated with trauma to the upper tarsal plate. Contact lens wear is the primary contributor, although an exposed suture following a corneal transplant, a foreign body or ocular prosthesis also could induce GPC [497]. Symptoms of GPC and DED can overlap, including discomfort, decreased contact lens wear time and mucin discharge. The key differentiating findings include large upper tarsal papillae and hyperemia with usually minimal corneal or bulbar conjunctival involvement [497]. Further, in most instances, the cause of the trauma usually is identifiable.

Atopic keratoconjunctivitis (AKC) is a chronic and potentially severe, visually threatening form of allergic eye disease. As AKC is a bilateral, chronic, inflammatory disease, the signs and symptoms may be similar, and DED may actually be present in many of these patients. Additionally, signs of inflammation are noted on the cornea, conjunctiva and eyelids. Common symptoms include photophobia, burning, tearing, itching, mucoid discharge, and eyelid hyperemia and hypertrophy, often with greater lower eyelid involvement. Some of the more common signs that are found in both AKC and DED include SPK, conjunctival injection or hyperemia, blepharitis/MGD and tear dysfunction [498–501]. The OSD in AKC patients is characterized by greater epithelial damage and SPK [502]. Prolonged inflammation plays an important role in the progression of OSD in patients with longstanding, active AKC [415]. The hallmark findings of AKC that may help differentiate it from DED include conjunctivitis (potentially cicatrizing), periorbital eczema [503], corneal neovascularization that could lead to eventual conjunctivalization of the cornea, symblepharon, keratoconus and anterior polar cataracts [504,505]. Other key findings that may aid in the differential diagnosis include a strong family history of multiple allergies, atopic dermatitis, the presence of asthma and periorbital eczema [506]. In fact, it is estimated that atopic dermatitis and asthma are present in 95% and 87% of AKC patients, respectively [497].

Vernal keratoconjunctivitis (VKC) causes rapid fluorescein breakup time, SPK associated with sodium fluorescein staining and increased conjunctival lissamine green staining [507]. Patients with VKC often report severe symptoms, including intense itching, burning, epiphora, conjunctival injection and photophobia [508,509]. Clinically, VKC is associated with the presence of large cobblestone papillae and/or Horner-Trantas dots [510]. The condition can lead to debilitating corneal damage, including shield ulcers and scarring. Another key differentiator from DED is that this condition tends to occur in younger male patients—most notably those under age 18 [511].

9.1.2. Viral conjunctivitis

Viral conjunctivitis is a relatively common presentation that affects patients of all ages, including the ages during which DED is most frequent. The majority of viral conjunctivitis cases involve the highly contagious adenovirus (65–90%) [512]. Adenovirus is capable of surviving for long periods on environmental surfaces and takes a long time to shed, giving it an incubation period of 4–10 days before it is clinically observable [513]. In addition to the two types of adenovirus; pharyngoconjunctival fever (PCF) and epidemic keratoconjunctivitis (EKC), other viral conjunctivitis causes include herpes viruses, picornaviruses, and several systemic viral infections.

Although viral conjunctivitis has a number of findings in common with DED, such as tearing, burning, redness, irritation, photophobia and blurred vision, a number of differentiating factors also exist. Patients with viral conjunctivitis usually experience redness and irritation in one eye initially, often spreading to the fellow eye within a few days. When asked, patients also often report recent upper respiratory tract infection or close contact with someone with a red eye. Morning crusting is also common. Exam findings usually reveal a watery, mucoid discharge and red, edematous lids. Preauricular lymphadenopathy is also commonly present [514].

The term EKC is used when adenoviral eye infections invade the cornea. EKC, in particular, tends to be accompanied by periorbital edema and significant inflammation that may also involve the extraocular muscles. A follicular response is often noted on the palpebral conjunctiva. Early stage EKC presents with positive preauricular lymphadenopathy on the ipsilateral side to the eye that first manifested the conjunctivitis. Approximately one week later, the cornea typically exhibits sub-epithelial infiltrates, which account for symptoms of irritation and pain, often leading to decreased visual acuity that can last months or even years after the infection subsides [513].

PCF is a highly infectious illness with systemic symptoms including sweats, sore throat, fever and headache. Myalgia, malaise, pharyngitis, and gastrointestinal disturbances also are typical in patients with PCF. Upper respiratory tract symptoms may precede ocular findings, but not in all cases. Acute follicular conjunctivitis and regional lymphoid hyperplasia with tender, enlarged preauricular lymph nodes are often also found in patients with PCF. PCF is most commonly observed in children and in groups living in close quarters, such as schools, prisons, ships, military bases and families. It is self-limiting and often dissipates within a week [514].

Herpes viruses that cause conjunctivitis include the herpes simplex virus, varicella-zoster virus, which also causes chickenpox and shingles, and Epstein-Barr virus, which also causes infectious mononucleosis. Herpes simplex virus in its primary form typically affects children and presents as a unilateral red eye. It is sometimes accompanied by a vesicular rash around the eyelid area. In the absence of ulceration or vesicles, herpes infection can be more difficult to diagnose. Secondary herpes simplex virus forms typically involve some form of keratitis in addition to the conjunctivitis. Interestingly, research suggests that dry eye is a stressor that may contribute to stromal keratitis in patients who have herpes simplex [515]. Herpes zoster conjunctivitis is also unilateral and typically is accompanied by a rash that involves pustules, vesicles and edema/hyperemia of the surrounding skin, respecting the midline. Conjunctivitis sometimes precedes the appearance of lesions, making diagnosis more challenging in patients with this inflammatory condition [516].

The Epstein-Barr virus infects >90% of the population [517]. Initial exposure generally occurs during infancy or early childhood and produces subclinical infection. However, if exposure occurs in adolescence, it often manifests as infectious mononucleosis.

Epstein-Barr virus infection of ocular structures most often results in transient follicular conjunctivitis [518] but can also manifest as DED, keratitis, uveitis, choroiditis, retinitis, oculoglandular syndrome, papillitis, and ophthalmoplegia [519]. Picornaviruses, such as enterovirus 70 and coxsackievirus A24, are highly contagious and often are the cause of epidemics. Like adenoviral conjunctivitis, picornaviruses cause an acute hemorrhagic response, although the clinical appearance is usually more severe. A number of systemic viruses—including as rubeola (measles), rubella (German measles), mumps, and influenza also frequently involve conjunctival infection [514]. In cases where clarification is desired, diagnostic tests with high sensitivity and specificity can help identify forms of viral conjunctivitis in minutes [520].

9.1.3. Bacterial conjunctivitis

Acute bacterial conjunctivitis is less common than viral and allergic conjunctivitis, but also shares several findings in common with DED. Bacterial conjunctivitis can affect patients of any age, but is most commonly found in children [521]. In adults, the more common culprits are gram-positive organisms such as *staphylococcus*, while in children bacterial conjunctivitis tends to be caused by *Haemophilus influenzae* and *streptococcus* species, with more than one causative organism in some cases [521]. As with DED, patients who have bacterial conjunctivitis may complain of irritation, foreign body sensation, burning, stinging and photophobia. However, they are often most concerned with the redness and discharge. Symptoms of bacterial conjunctivitis usually include a greater degree of conjunctival injection compared to conjunctivitis caused by viruses or DED. Also the discharge is wet and mucopurulent, rather than dry and crusty, and patients often complain of matting or adherence of the eyelids, especially in the morning. Bacterial conjunctivitis can be can be unilateral or bilateral and can sometimes be accompanied by systemic findings, especially in children. Systemic symptoms might include fever, malaise, purulent rhinorrhea and a respiratory infection. Otitis media is also common in children and is highly indicative of *H. influenzae* infection [522]. In some cases, bacterial conjunctivitis is accompanied by a red sheen around the eyelids, which is indicative of preseptal cellulitis.

9.2. Anterior blepharitis

Inflammation of the eyelids can result from infection by, or allergic reaction to, external agents. The clinical features of blepharitis include redness, exanthema, sores, eschar, swelling, and bullous formation. Blepharitis is classified according to its anatomic location. Anterior blepharitis affects the base of the eyelashes, eyelash follicles, and/or eyelid skin. Inflammation of follicles is categorized as marginal blepharitis, whereas that of eyelid skin is blepharo-dermatitis. The pathogenesis of anterior blepharitis is infectious or noninfectious in nature, and so the location and cause of the condition should be considered for diagnosis [523]. Clinical features of anterior blepharitis often overlap those of DED [524]. Recurrent or persistent blepharitis can cause DED, thus observation of the eyelid is important for adequate diagnosis of DED. The tear meniscus, tear film breakup time and pattern, foamy discharge and debris in the tear film should be observed [524], along with the eyelid position (i.e., ectropion and entropion), eyelid closure (i.e., lagophthalmos), blink response and the anterior eyelid margin (noting any collarettes around eyelashes). Staphylococcal or seborrheic anterior blepharitis are linked to ADDE [482,524] in 50–75% of cases [525,526], perhaps due to the decreased tear volume supporting less lysozyme or immunoglobulins [526]. Definitive diagnosis is made by identification of the responsible microorganism or allergen. There are no specific clinical diagnostic tests for blepharitis. However, cultures of the eyelid margins may be indicated for patients who have recurrent anterior blepharitis with severe inflammation as well as for patients who are not responding to therapy [524].

9.3. Demodex

Demodex mites are common elongated microscopic ectoparasites that live on the surface of the human body. Demodex infestation is related to age with 84% of the population at age 60 and 100% of those older than 70 years exhibiting Demodex infestation [527]. Demodex can spread from the face to the eyelids, perhaps leading to blepharitis and also rosacea [527–530], which may be the link between DED and meibomian gland dysfunction [528,531–533]. However Demodex infestation can also be found in asymptomatic patients [529]. Contact lens wearers do not show higher rates of Demodex infestation than non-wearers, but the relationship with DED symptoms and signs has not been investigated [534]. Two species, *Demodex folliculorum* and *Demodex brevis* have been identified in human eyelids [529,535,536]. *Demodex folliculorum* are typically found in the lash follicles of the eyelids, whereas *Demodex brevis* burrow deep into sebaceous and meibomian glands. Sebum is thought to be their main food source and Demodex mites may consume follicular and glandular epithelial cells, which may lead to direct damage of the lid margin [529]. Demodex mites can cause blepharitis by carrying bacteria on their surface including *streptococci* and *staphylococci* [529,537]. Also the protein inside the Demodex mites and their waste products may trigger inflammatory responses, likely via a delayed hypersensitivity or an innate immune response [538]. Demodex-based lid margin inflammation may result in blepharoconjunctivitis [529]. Proper treatment of ocular demodicosis may resolve blepharoconjunctivitis in adults [529,539], however its role in children remains unclear [529]. Severe cases of demodex with inflamed lid margins can affect the cornea [529,540].

Demodex can sometimes be observed *in situ* with high magnification slit lamp microscopy, on epilated lashes using standard light microscopy or using more advanced techniques, such as IVCM [329,440,528,529,541]. Liu et al. [529] recommend the following clinical procedure based on a comprehensive literature review:

1. Clinical history: high index of suspicion when blepharitis, conjunctivitis or keratitis in adult patients or blepharoconjunctivitis or recurrent chalazia in young patients are refractory to conventional treatments, or when there is madarosis or recurrent trichiasis.
2. Slit-lamp examination: typical cylindrical dandruff at the root of eyelashes.
3. Microscopic confirmation: detection and counting of Demodex eggs, lavae and adult mites on epilated lashes.

To avoid epilating eyelashes it has also been reported that Demodex leave the follicle and are visible by slit lamp microscopy after gentle tension is applied to the lash and the lash manually rotated with forceps, encouraging exodus of the mites and allowing the lash to “scrape out” Demodex deep within the follicle [542]. As Demodex infestation can also occur in non-DED patients [527], its diagnostic contribution is limited.

9.4. Parasitic infections

Chlamydia is an obligate intracellular parasite and one of the most common sexually transmitted infections [543]. Trachoma (or

granular conjunctivitis) is caused by chlamydia trachomatis which results in inflammation, corneal inflammation and scarring of the conjunctiva, obliterating the meibomian gland ductules and goblet cells, and inducing DED complications [544]. A genital infection with chalmydia trachomatis is also the main predisposing factor for adult inclusion conjunctivitis, which is most common in young adults who are usually asymptomatic. The key differential signs from typical DED include the generally unilateral infectious nature, which can be accompanied by corneal ulcers, subepithelial infiltrates or opacity, superior epithelial keratitis, superior pannus, conjunctival scarring, mucopurlent discharge and follicles.

9.5. Corneal and conjunctival abnormalities

The corneal epithelial barrier can be compromised in the setting of DED, and manifest clinically as punctate epithelial keratopathy/erosions by fluorescein staining, most prominently in the interpalpebral zone. Other epithelial changes in DED can include filaments, epithelial ridges and, in late stages, keratinization. The epithelial barrier integrity, however, can be compromised due to other non-DED etiologies, which can also lead to epithelial changes and corneal fluorescein staining (Table 7). These conditions often co-exist with DED and may contribute to the OSD. It can sometimes be challenging to determine whether the main underlying reason for the epithelial disease is DED, another etiology, or both.

Certain clinical features help to distinguish these other epithelial abnormalities from those that are directly related to the loss of tear film homeostasis. The history is sometimes helpful; particularly, patients who might have a history of contact lens wear [545], use of multiple eye drops or exposure to toxic chemical agents [495,546]. More importantly, the clinical examination often provides additional information to alert the clinician. Specifically, the pattern and location of the epithelial changes (particularly fluorescein staining) can provide critical diagnostic clues that help distinguish DED from other alternative (or concomitant) conditions affecting the corneal epithelium. For instance, fluorescein staining in a “whorl” pattern can be seen in the setting of epithelial stress (such as toxicity from medications) [495] or conjunctivalization of the cornea due to limbal stem cell deficiency [545,547]. Likewise, fluorescein staining in the superior cornea, which is not typical for DED, may be seen in conditions such as superior limbic keratoconjunctivitis [548], floppy eyelid syndrome [549], and contact lens wear [545].

Conjunctival disease may be another co-morbid condition in patients with DED. One important disorder that can symptomatically mimic DED, and often co-exist and contribute to the patient's tear film instability, is conjunctivochalasis [550,551]. In addition to the clinical findings, the lack of response to standard DED therapies further raises the suspicion and the need to address this co-existing condition. Other critical signs of co-existing conjunctival disease are cicatricial changes (sub-epithelial scarring, fornix foreshortening, cicatricial entropion/trichiasis, and in later stages symblepharon and keratinization) [380,552]. These findings may be a manifestation of underlying systemic diseases such as mucous membrane pemphigoid (also known as ocular cicatricial pemphigoid) and chronic Stevens-Johnson syndrome [552–554]. While these conditions universally have dry eyes as part of the clinical picture, an early diagnosis is critical, as the management often requires more advanced therapies including systemic immunomodulatory therapy.

9.6. Filamentary and other keratitis, and keratopathies

Filamentary keratitis is generally a chronic corneal condition, characterized by fine strands of degenerated epithelial cells and mucus attached to the cornea at one or both ends [555]. Patients often experience foreign body sensation, grittiness, discomfort, photophobia, blepharospasm, and increased blinking. ADDE is the most common ocular condition associated with filamentary keratitis and best-practice management involves treating the underlying DED and potential mechanical removal of the corneal filaments [556]. Interstitial keratitis is any non-ulcerating inflammation of the corneal stroma, often with vascularisation, but without involvement of either the epithelium or endothelium. The underlying causes are generally infectious or immune-mediated [557]. Neurotrophic keratitis from dysfunction of the ophthalmic division of the trigeminal nerve caused by conditions such as diabetes mellitus, ocular herpes simplex, neoplasia, and ophthalmic surgery is associated with reduced aqueous production [558]. However, treatment after the early stages of the disease can require more radical treatment than primary DED such as antibiotics, antivirals, autologous serum and steroids [559]. Bullous keratopathy is a pathological condition in which small vesicles, or bullae, form in the cornea due to endothelial dysfunction. These blister-like formations can undergo painful ruptures and disrupt vision. Treatments include hyperosmotic eye drops to reduce swelling (5% sodium chloride), amniotic membranes, bandage contact lenses to reduce discomfort, antiglaucoma medications to reduce the flow of fluid into the cornea, and corneal transplantation to replace the damaged tissue [560]. Hence while filamentary and other keratitis, and keratopathies can mimic some of the signs of DED, slit lamp detection of vascularisation, anterior chamber cells and flare, and stromal edema generally set them apart from primary DED.

9.7. Rheumatological conditions

Eye involvement is common in patients with systemic autoimmune diseases, particularly rheumatoid arthritis, Sjögren syndrome, seronegative spondyloarthropathy, and antineutrophil cytoplasmic antibody-associated vasculitis. The eye is a privileged immune site, but commensal bacteria are found on the ocular surface. Eye injury may be inflammatory, vascular or infectious, as well as iatrogenic, but DED can also be a presenting symptom. Over half of newly presenting DED cases to a tertiary centre were

Table 7
Common causes of corneal epithelial abnormalities.

Epithelial Trauma	lid margin keratinization, trichiasis/entropion, foreign body, superior limbic keratoconjunctivitis, floppy eyelid, contact lens wear (including hypoxia)
Epithelial Toxicity	preservatives from topical medications; such as glaucoma drops, vidarabine; mitomycin-C; fluorouracil (5-FU); other chemical/environmental exposure
Limbal Stem Cell Disease	autoimmune diseases (Stevens-Johnson syndrome, mucous membrane pemphigoid), contact lens wear, chemical injury, aniridia, ectodermal dysplasia
Epithelial Dystrophies	epithelial basement membrane dystrophy. Meesman's dystrophy
Conjunctival Scarring	mucous membrane pemphigoid, chronic Stevens-Johnson syndrome, chronic atopic keratoconjunctivitis

secondary to a known (48%) or undiagnosed (5%) inflammatory disease, primary thyroid disorder, Sjögren syndrome or rheumatoid arthritis [561]. Sjögren syndrome is considered a sub-classification of DED [380], but requires specific diagnostic differentiation from other forms of DED to facilitate appropriate interdisciplinary treatment and allow monitoring of potentially life-threatening complications. Unfortunately the average time to diagnose primary Sjögren syndrome from symptom onset is 6.5 years [562], despite being an independent risk factor for non-Hodgkin lymphoma [563], and the most highly associated risk factor among all rheumatic diseases for malignancy [487]. The revised international classification criteria for Sjögren syndrome, by the American-European Consensus Group Criteria, 2002 [245,564] includes one criterion of daily feeling of dry mouth for more than 3 months, recurrent or persistent swollen salivary glands as an adult, or a need to drink liquids to aid swallowing dry food, thus any of these symptoms in a patient reporting DED should instigate a referral. There are also now serological biomarker tests for Sjögren syndrome [565]. It should be noted that tests not recommended for the diagnosis of DED, such as the Schirmer test, are still recommended for the diagnosis of Sjögren syndrome [245].

9.8. Lid related disease

Lid related disease such as chalazion or infectious hordeolum, may result in DED symptoms. Other eyelid conditions such as anterior blepharitis and MGD can inform the management of DED and therefore the eyelid should always be carefully observed when DED is investigated.

9.9. Visual asthenopia

General symptoms of visual discomfort may include those linked to DED [566]. DED is the predominant cause of computer vision syndrome [567], resulting in the reporting of general visual symptoms after prolonged use of digital screens compared to equivalent paper copy tasks [568]. Incomplete blinks rather than a reduction in blink rate appears to be associated with these symptoms [569]. Differentiation from primary DED is on the basis of history informed triggers of dryness and more general symptoms such as the eyes being tired, hurting, feeling heavy, burning, straining, stinging and experiencing photophobia [91].

9.10. Graft versus host disease (GVHD)

GVHD is an immune-mediated inflammatory disease that occurs following allogeneic hematological stem cell transplantation and causes destruction of host tissues by immunocompetent cells from the donor. Typical ocular complications in the acute form of the condition are pseudomembranous conjunctivitis and acute hemorrhagic conjunctivitis in 12–17% of cases [570,571], whereas 60–90% with the chronic form develop ocular symptoms of DED [572], perhaps due to tear fluid levels of receptor agonist IL-8/CXCL8 and interferon inducible protein IP-10/CXCL10 [28]. Ocular symptoms can be minimised by a stepwise approach to treatment involving topical anti-inflammatory medications and autologous serum tears, but patients must be monitored closely, as they are prone to serious ocular complications such as corneal perforation and endophthalmitis [573].

9.11. Contact lenses

Contact lenses can induce dry eyes (termed CLIDE) and appropriate management strategies should be employed to minimize these [495,574]. This should be distinguished from people who have diagnosed primary DED and wish to wear contact lenses where, as well as the selection of lens modality and material, non-preserved DED treatments should be considered [377].

9.12. Psychological factors

Concomitant psychosocial issues have been associated with DED. Patients with DED have been shown to have increased prevalence of sleep and mood disorders [575]. Anxiety and depression have also been reported with increased frequency in DED patients in a variety of studies [576–578]. In one population-based cross-sectional study, of over 6000 women, these findings were similarly confirmed. Subjects with a diagnosis of DED were more likely to experience severe psychological stress [odds ratio (OR) 2.5], depressive mood [OR 1.5], and anxiety [OR 1.5] [579]. In another large series of over 7000 DED patients, the adjusted OR of DED and anxiety was 2.8 and DED and the OR for depression was 2.9 [580]. Beyond depression and anxiety, it has been suggested that DED can lead to neuropathic ocular pain and this has been shown to occur with greater frequency in patients who also have comorbid chronic pain syndromes [333,581]. Post-traumatic stress disorder has also been associated with DED and may have a link via treatment medication use or the underlying disease process [61,582]. Neuropathic pain can be differentiated from a disease mechanism through the use of anaesthetic [583], although this has not been reported in relation to DED symptoms.

Specialized forms of DED, such as Sjögren syndrome, has been associated with cognitive and mood disorders [584]. Signs of these disorders signify central nervous system involvement, which is an emerging area within Sjögren syndrome understanding. Other studies have noted that patients with Sjögren syndrome self-report greater fatigue and depression, however when compared to matched controls showed no greater dysfunction on objective tests of cognition and psychomotor function [585]. Hence, a patient's perception of disease and function can be powerful. Health-related quality of life has been studied in Sjögren syndrome, showing that these patients often worry about the consequences of their illness [586].

10. Emerging technologies

Lab-on-a-chip systems capable of evaluating multiple biomarkers simultaneously are being developed by several companies and hold promise for the differential diagnosis of DED as well as systemic diseases [587]. While regulators to date have shown reluctance in approving diagnostic panels in the case of OSD, the availability of these technologies are anticipated to be of transformative value to the ophthalmic communities. Future developments will include the creation of a multiplex tear assay device that incorporates the collection and handling of sub-microliter amounts of tear [588,589]. Since ocular surface oxidative stress is an important trigger of inflammation [590], another exciting development would be the evaluation of diagnostic tools for the assessment of reactive oxygen species or oxidised products in DED. Technology is needed to determine key pathophysiological indicators of dry eye, such as osmolarity and inflammation, over the whole ocular surface in real-time within the inter-blink interval to better understand the predicated localized changes and how they impact DED [591].

An additional non-invasive assessment of tear film stability has been proposed by Varikooty et al. [592] Using this technique, tear film spread and stability is quantified through the measurement of tear film particle dynamics. Video recordings are made using a slit