Purpose of review
Dry eye disease (DED) is a complex, multifactorial condition that is challenging to diagnose and monitor clinically. To date, diagnosis has consisted largely of self-reported symptom questionnaires and a collection of clinical tests including vital dye staining, estimation of tear breakup time and Schirmer’s testing, as no gold standard exists. As the dry eye field has made progress in understanding disease pathogenesis, new methods for assessment of this condition have been developed.

Recent findings
DED is now known to be characterized by tear hyperosmolarity and ocular surface inflammation, and there are now commercially available devices that accurately and reliably measure tear osmolarity and matrix metalloproteinase 9, a marker of inflammation and tissue breakdown. In addition, there are a variety of imaging modalities that have shown promise in their ability to identify patients with DED by assessing tear film dimensions and tear film instability.

Summary
There is a significant need for the development of tear film assessments for accurate diagnosis and monitoring of dry eye. There are a number of new devices and techniques that have shown promise in their ability help clinicians manage patients with DED.

Keywords
dry eye disease, matrix metalloproteinase 9, tear film, tear osmolarity

INTRODUCTION
Dry eye disease (DED) is an immunoinflammatory disorder of the tear film and ocular surface and is one of the most common ophthalmic conditions for which patients present to healthcare providers [1]. It is characterized by symptoms of dryness, visual instability, foreign body sensation and irritation, all of which have a significant negative impact on patient quality of life [2,3]. Although the mechanisms underlying DED continue to be investigated, there is evidence to suggest that dysfunction of both the innate and adaptive immune responses contribute to DED pathophysiology.

The complex and multifactorial nature of DED, along with the fact that patient signs and symptoms do not always correlate, have made the diagnosis and management of this disease challenging. In the absence of a gold standard diagnostic tool, physicians have to rely on a combination of clinical examination techniques and self-reported patient symptom questionnaires. Given the prevalence and impact of DED, there is a need for accurate and reliable methods to assess this condition. Assessments of the tear film in particular have attracted considerable attention as diagnostic tools because of the tear film’s accessibility and crucial role in maintaining ocular surface health and homeostasis.

TEAR FILM PHYSIOLOGY
The preocular tear film plays an important role in overall ocular health and function. It is approximately 7 μm thick and comprises lipid, aqueous and mucin layers. The outermost lipid layer is produced by the meibomian glands and serves to stabilize the tear film and slow evaporative losses [4]. The middle aqueous layer constitutes 90% of the tear film thickness and is produced by the lacrimal glands [4]. The innermost layer comprises mucins produced by conjunctival goblet cells and membrane-associated mucins produced by epithelial cells [5]. Together, these layers enable the tear film to perform multiple specialized functions including...
providing a uniform refractive surface, maintenance of the corneal and conjunctival epithelium, and innate host defense.

The tear film is dynamic, containing proteins such as IgA and IgG [6,7], a variety of electrolytes (sodium, potassium, calcium, magnesium, zinc, manganese, chloride, bicarbonate and phosphate) [8,9] and cytokines (IL-17, IL-6, IL-10, IL-4, IL-2, IFN-γ and TNF-α), many of which are upregulated in DED [6,10–13]. Optimal ocular health depends not only on the composition, but also on the production and distribution of the tear film, and these parameters are known to be imbalanced in a variety of ocular conditions, including DED [14,15]. In the ongoing effort to improve patient care, a number of diagnostic tests evaluating these parameters have been developed to help diagnose and monitor DED (Table 1).

**TEAR OSMOLARITY**

Tear hyperosmolality, resulting from decreased aqueous tear production and/or increased evaporation, is now recognized as a major contributing factor in DED pathogenesis. In mouse models of DED and corneal epithelial cell cultures, hyperosmolar stress leads to tear film instability [16], altered corneal nerve function [17,18] and initiation of multiple inflammatory pathways that lead to upregulation of inflammatory cytokines and matrix metalloproteinases (MMPs) with subsequent ocular surface damage [16,19–22].

Quantification of tear osmolarity has gained in popularity due to the fact that this measure provides a single numerical value that can be objectively monitored across office visits. Although multiple osmolarity cutoff values have been validated for the diagnosis of DED [23], an osmolarity greater than 308 mOsm/l is now commonly regarded as abnormal [24]. A difference of more than 10 mOsm/l between the two eyes is also suggestive of DED, as intereye variability increases with increasing DED severity [24]. The TearLab Osmolarity System (TearLab Corporation, San Diego, California, USA) is currently the only United States Food and Drug Administration (FDA)-approved commercially available in-office device for measuring osmolality. A number of studies using the TearLab device have shown that tear osmolarity is an effective tool for diagnosing DED and that osmolarity correlates well with disease severity [25].

Sullivan et al. [26] developed a composite index of disease severity based on the dry eye workshop grading scale and found tear osmolarity ($R = 0.74$) to have the strongest correlation with disease severity among variables assessed, including tear breakup time (TBUT, $R = 0.55$), Schirmer's test ($R = 0.41$), corneal fluorescein staining (CFS, $R = 0.66$) and ocular surface disease index (OSDI, $R = 0.64$, $R$ values calculated from provided $r^2$ values). Using a similar

**Table 1. Summary of tear film assessments for the diagnosis of dry eye**

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Description</th>
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<tbody>
<tr>
<td>TearLab</td>
<td>Commerially available point-of-care test measuring tear osmolarity, with elevated osmolarity ($&gt;308$ mOsm/l) and/or an intereye difference of 10 mOsm/l or more considered to indicate dry eye. High sensitivity and specificity for disease diagnosis. Correlates well with other signs of disease</td>
</tr>
<tr>
<td>InflammaDry</td>
<td>Commerially available point-of-care test indicating the presence or absence of MMP-9, a proinflammatory cytokine involved in tissue degradation and remodeling. High sensitivity and high specificity for disease diagnosis. Correlates well with other signs of disease</td>
</tr>
<tr>
<td>Wavefront aberrometry</td>
<td>Widespread imaging technology which has been used to quantify higher-order aberrations resulting from tear film irregularity and instability</td>
</tr>
<tr>
<td>Videokeratography</td>
<td>Imaging technology which has been used to automatically quantify TBUT, a measure of tear film instability</td>
</tr>
<tr>
<td>OCT</td>
<td>Widespread imaging technology that has been used to measure tear meniscus dimensions including radius, height and cross-sectional area, all of which have been shown to be significantly decreased in dry eye</td>
</tr>
<tr>
<td>LipiView</td>
<td>Commerially available imaging device that uses interferometry to measure lipid layer thickness, an indicator of meibomian gland function</td>
</tr>
</tbody>
</table>

MMP-9, matrix metalloproteinase 9; OCT, optical coherence tomography; TBUT, tear breakup time.
index of disease severity, Lemp et al. [24] found that at a single office visit, tear osmolarity had both a high sensitivity and specificity for the diagnosis of mild, moderate and severe DED. They found tear osmolarity to have 72.8% sensitivity and 92.0% specificity, and none of the other measured variables, including TBUT (84.4% sensitivity and 45.3% specificity), CFS (54.0% sensitivity and 89.3% specificity) and Schirmer’s test (79.5% sensitivity and 50.7% specificity), surpassed tear osmolarity in both sensitivity and specificity.

Studies have also shown that tear osmolarity generally correlates well with other measures of DED in conditions characterized by ocular surface disease. In a cohort of ocular graft-versus-host disease patients, tear osmolarity showed strong correlation with TBUT ($R = 0.681$) and moderate correlation with Schirmer’s test ($R = -0.476$) and OSDI ($R = -0.455$) [27]. In a similar study [28] in patients with ocular mucous membrane pemphigoid, tear osmolarity was found to be strongly correlated with TBUT ($R = 0.80$). Versura et al. [29] also found that in patients with severe dry eye, tear osmolarity showed moderate correlation with TBUT ($R = -0.558$) and Schirmer’s test ($R = -0.473$).

Still, the exact role of tear osmolarity as a diagnostic tool for DED in practice remains to be seen. In contrast to the above studies, there are others demonstrating a lack of correlation between tear osmolarity and both signs and symptoms of disease. In a study evaluating the change in tear osmolarity, CFS and OSDI scores in known DED patients across multiple office visits, Amparo et al. [30] found a lack of correlation between tear osmolarity and fluorescein staining ($R = -0.02$) and between tear osmolarity and OSDI ($R = -0.091$). There are also studies [31,32] demonstrating overlap in tear osmolarity values amongst normal and DED patients. This suggests an inherent degree of variability in tear osmolarity, and indeed variability is known to increase with disease severity [24,33]. The variability in tear osmolarity seen in these studies may be a reflection of the multifactorial and intermittent nature of DED, and emphasizes that our understanding of tear osmolarity as a diagnostic tool continues to evolve.

**MATRIX METALLOPROTEINASE 9**

MMP-9 is a proteolytic enzyme involved in normal and pathologic tissue remodeling. As a proteinase that acts on extracellular matrix and cell surface adhesion molecules, it serves to degrade tissue and facilitate leukocyte migration. It has also been shown to regulate cellular activity and can contribute to inflammation by activating cytokines [34,35]. MMP-9 plays an important role in normal tissue remodeling, such as in wound healing, in which expression is tightly regulated. However, it is also known to be upregulated in a number of pathologic processes in which dysregulation and elevated levels indicate ongoing inflammation and tissue damage [35].

Multiple studies have demonstrated a significant upregulation of MMP-9 in animal models of DED [36,37], and the functional significance of this enzyme has been demonstrated through use of MMP-9 knockout mice, which do not develop corneal epitheliopathy despite exposure to a dry eye model [38]. Clinically, MMP-9 levels are known to be significantly upregulated in the tear film of patients with DED as compared with normal controls [39–41], and tear film MMP-9 levels have been found to correlate with clinical signs of DED including Schirmer’s test, tear osmolarity as well as symptoms of DED, as measured by the OSDI questionnaire [40].

These findings provided the rationale for development of a means to detect MMP-9 in the clinic for the diagnosis of DED. The InflammaDry test (RPS, Inc., Sarasota, Florida, USA) is currently the only FDA-approved point-of-care device for the detection of MMP-9 in patients. Using a hand-held immunoassay device, it indicates whether or not MMP-9 is present in a tear sample, with a threshold concentration of 40 ng/ml. One possible shortcoming of the InflammaDry device is the binary nature of its read-out, as it gives a ‘positive or negative’ reading that may limit the ability to monitor disease over time. A finding to note, however, is that as an immunoassay, the intensity of the positive read-out on the InflammaDry may reflect the concentration of MMP-9, although this may be difficult to quantify.

Several studies using InflammaDry have shown promise for reliable DED detection. In a prospective, multicenter trial, Sambursky et al. used the InflammaDry to evaluate 63 normal controls and 143 DED patients, with DED diagnosed if a patient had all of the following: an OSDI of 13 or higher, Schirmer’s test less than 10 mm in 5 min, TBUT less than 10 s and the presence of CFS. They found the device had 85% sensitivity and 94% specificity, with a 97% positive predictive value and 73% negative predictive value [42]. In a similar prospective trial, MMP-9 demonstrated a high level of concordance with clinical markers of disease severity (TBUT, Schirmer’s test and CFS) and symptoms as measured by the OSDI [43*].

As indicated by its high sensitivity and specificity, MMP-9 detection tests (specifically InflammaDry) show promise as a tool for DED diagnosis; however, two studies recently found low correlation
between MMP-9 and signs and symptoms of disease. Lanza et al. evaluated 128 patients using a number of clinical markers including MMP-9 concentration using the InflammaDry, and patients were divided on the basis of whether they tested positive or negative for MMP-9. They found that these two groups did not differ with respect to any of the measured clinical signs of DED, including TBUT, CFS, Schirmer’s test and corneal pain threshold [44]. Similarly, Schargus et al. [45] evaluated 20 elderly patients for MMP-9 concentration, tear osmolarity and clinical signs of disease and found no statistically significant correlation between MMP-9 concentration and TBUT, Schirmer’s test, CFS and OSDI. Given that MMP-9 is a marker of tissue breakdown and inflammation, the findings in these studies may highlight the need to factor disease severity and chronicity into the interpretation of the test, as well as the underlying etiology of the clinical picture of DED.

**TEAR FILM ASSESSMENT BY IMAGING**

In the ongoing effort for novel diagnostics for DED, a number of imaging techniques have been described as useful methods for the assessment of the tear film in DED. These include wavefront aberrometry, videokeratography, anterior segment optical coherence tomography (OCT) and interferometry. These techniques have the advantage of offering noninvasive, quantitative and objective assessments of the tear film.

Wavefront aberrometry indirectly measures tear film stability by quantifying higher-order aberrations, which result from irregularities in the air–tear interface, such as may occur in DED. Higher-order aberrations have been shown to be significantly greater in DED patients as compared with normal controls, and may be a useful method for diagnosing DED patients [46,47]. Videokeratography can also be used to obtain an automated TBUT. Downie [48] recently demonstrated 82% sensitivity and 94% specificity for the diagnosis of DED, whereas using high-speed placido disc imaging to assess tear film breakup time.

OCT has been used to accurately quantify the tear meniscus, and tear meniscus radius, height and cross-sectional area have all been shown to be significantly smaller in DED patients compared with normal controls [49]. Tear meniscus area is also known to correlate well with symptoms of dry eye and Schirmer’s test [50]. OCT can also be used to measure corneal and conjunctival epithelial thickness that have been shown to vary in DED. Kanellopoulos and Asimellis [51] have shown increased central corneal epithelial thickness in DED patients, whereas more recently Liang et al. have shown thinner limbal epithelium and thicker bulbar conjunctival epithelium in DED patients, both of which correlated with OSDI symptoms and clinical signs of disease.

Finally, interferometry has been used to quantitatively evaluate lipid layer thickness in the tear film. Using the LipiView interferometer (TearScience Inc, Morrisville, North Carolina, USA), Finis et al. [52] demonstrated that lipid layer thickness has a sensitivity of 65.8% and a specificity of 63.4% for detecting meibomian gland dysfunction, one of the major causes of evaporative type DED.

**CONCLUSION**

DED is a multifactorial disorder of the ocular surface that is one of the most common ophthalmic conditions affecting patients. The diagnosis of DED has been complicated by the lack of a gold standard diagnostic tool and the fact that the symptoms and signs of the disease do not always correlate. To date, the diagnosis and assessment of DED has been based on patients’ reported symptoms and interpretation of a collection of clinical signs of disease.

As our understanding of DED evolves, knowledge of the underlying mechanisms of the disease has provided new strategies for diagnosis. In particular, the recognition of tear hyperosmolarity and ocular surface inflammation as key components of disease pathogenesis has led to the development of point-of-care tear film diagnostics. Quantifying tear osmolarity and detecting MMP-9 have shown promise as diagnostic tools, and their increased use will hopefully provide more insight into optimal DED management going forward.

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**Conflicts of interest**


**REFERENCES AND RECOMMENDED READING**

Papers of particular interest, published within the annual period of review, have been highlighted as such: ● of special interest ●● of outstanding interest

Tear film assessments for the diagnosis of dry eye

Dohlman et al.