The tear ferning test: a simple clinical technique to evaluate the ocular tear film

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A healthy tear film is very important for many major functions of the ocular surface. Dry eye disease is a significant clinical problem that needs to be solved, but the poor correlation between clinical signs and reported symptoms makes it difficult for the clinician to apply a scientific evidence basis to his clinical management. The problem is compounded by the difficulties of evaluating the tear film due to its transparency, small volume and complex composition. Practical insight into tear film composition would be very useful to the clinician for patient diagnosis and treatment, but detailed analysis is restricted to expensive, laboratory-based systems. There is a pressing need for a simple test. The tear ferning test is a laboratory test, but it has the potential to be applied in the clinic setting to investigate the tear film in a simple way. Drying a small sample of tear fluid onto a clean, glass microscope slide produces a characteristic crystallisation pattern, described as a “tear fern”. This test is currently not widely used because of some limitations that need to be overcome, but several studies have demonstrated its potential. Such limitations need to be resolved so that tear ferning could be used in clinic setting to assess the tear film.

Keywords: tear film, dry eye, ferning, diagnosis, clinical test
THE TEAR FILM

The pre-ocular tear film is a thin, complex and moist layer, which covers the cornea and bulbar and palpebral conjunctivae.\(^1\) It is frequently described as a tri-laminar structure that contains layers, of complex chemistry, of a superficial lipid layer, an intermediate aqueous layer, and an underlying mucous layer.\(^2,3\) The composition and structure of the tear film change with eye closure, age, conjunctival stimulation, tear flow rate\(^4\) and ocular surface disease.\(^5\)

The superficial lipid layer is thin and oily,\(^6\) around 0.1 μm thick,\(^7\) and derived mainly from the tubuloacinar meibomian glands in the upper and lower eyelids.\(^8\) It contains a mixture of triacylglycerols, fatty acids and free sterols.\(^9\) It is believed that such layer has two phases. The outer phase is thick and contains non-polar lipids such as hydrocarbons, triglycerides, sterol esters and wax esters, while the inner phase is thin and contains polar, lipids such as phospholipids.\(^5,10\) Distributing and spreading of the lipid layer occurs with eyelid blinking. The main function of the lipid layer is to prevent evaporation of the aqueous phase from the pre-ocular surface.\(^2\) Also, it prevents contamination of the ocular surface tear film by polar lipids of the skin surrounding the palpebral aperture.\(^11\)

According to Wolff’s\(^2\) model, the aqueous layer is the major constituent of the tear film and composed of aqueous tears with a thickness of 6 to 10 μm.\(^7\) Its components include numerous electrolytes, proteins, vitamins, peptide growth factors, hormones, antimicrobials, immunoglobulins and cytokines. These components protect the ocular surface, maintain the tear film structure, provide ocular defence against infection agents, moderate osmolality of the tears and working as a buffer to maintain the pH. Also, they transmit
messages between the epithelial ocular surface tissues by lowering the tension of the ocular surface and allow the tear film to spread smoothly.\textsuperscript{1,5,12,13} The aqueous layer originates from the main lacrimal gland, the accessory glands of Wolfring, and the accessory glands of Krause.\textsuperscript{14} There are more than 60 different proteins, mainly secreted by the acinar cells of the lacrimal glands, have been detected in the human tear film.\textsuperscript{15} The total tear protein concentration measured experimentally is highly dependent on the type of collection method used.\textsuperscript{16} For example, 6–20 g/l has been found with non-stimulated tears using a capillary pipette, whereas 3–7 g/l have been found with stimulated tears.\textsuperscript{17,18} The main proteins of the tear film are lysozyme, lactoferrin, lipocalin, and immunoglobulin A (sIgA), which are secreted in response to an intracellular stimulus.\textsuperscript{20,21} Other proteins reported to occur in non-stimulated tears such as serum albumin, IgG, ceruloplasmin, transferrin, and monomeric IgA.\textsuperscript{5}

The tear film aqueous layer contains a large variety of ions, including sodium, potassium, magnesium, calcium, chloride, bicarbonate and phosphate ions. These ions are highly influential on the osmolality of tears\textsuperscript{12,13} and have an important role in the maintenance of epithelial integrity.\textsuperscript{21} They also work as a buffer to maintain the pH.\textsuperscript{22} The electrolyte concentration is very important, because a high concentration is a feature of dry eye,\textsuperscript{23} and could cause damage to the ocular surface.\textsuperscript{5} The sodium and potassium ions are the main positively-charged ions (cations) in tears, while chloride and bicarbonate are the main negative ions (anions). Sodium is the main cationic constituent of the aqueous and vitreous humour, and while the concentration of potassium in the cornea is much less than sodium, sodium and potassium both play an essential role in osmotic regulation. Any
change in the sodium level produces an opposite change in the potassium level. Chloride and bicarbonate ions regulate the sodium and potassium ions concentration in order to buffer the tear film.

The mucous layer forms the posterior layer of the tear film, adjacent to the corneal epithelium, and contains mucins and inorganic salts suspended in water. Holly and Lemp contend that the mucous layer is not strictly a part of the aqueous fluid film since it is highly hydrated in a semi-solid state. The mucous layer has been described as a sponge which consists mainly of high molecular weight glycoproteins with a high carbohydrate-to-protein ratio. Ocular mucins are mainly secreted by the conjunctival goblet cells, with a small proportion secreted by the stratified epithelial cells of the ocular surface. Ocular mucous fulfils many functions, including lubrication and helping the eyelid margins and palpebral conjunctiva to slide smoothly during blinking and ocular movements. It also protects the cornea and conjunctiva from abrasion by covering foreign bodies with a slippery coating of mucous and providing wetness to the ocular surface through the function of the ocular glycoproteins in glycocalyx formation. Mucins are classified into two types: trans-membrane and secretory mucins, with secretory mucins further subdivided into gel-forming and soluble types. trans-membrane mucins are expressed by conjunctival and corneal epithelial cells gel-forming secretory mucins are secreted by the conjunctival goblet cells. Soluble secretory mucins are secreted by the lacrimal glands and conjunctiva.
DRY EYE

The quantity and quality of the tear film composition are important for maintaining the ocular surface and good vision. Absence or degeneration in the quantity and/or quality of the tear film leads to a chronic tear film problem and, ultimately, dry eye. Dry eye is a very common eye complaint throughout the world. Various studies indicated that up to 20% of adults aged 45 years or more are believed to suffer from dry eye symptoms. In the elderly, dry eye is amongst the most common ocular abnormalities, and is encountered daily in optometric practice around the world.

The Subcommittee of the International Dry Eye Workshop revised the definition of dry eye to be: “Dry eye is a multi-factorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface”.

Although it is well established that there are different sub-types of dry eye, it is not known if tear ferning can differentiate between aqueous deficient and evaporative dry eye. In this paper, the term ‘dry eye’ has been used to describe all types of dry eye.

The causes of dry eye are multi-factorial and can be related to loss of function of the tear film and the ocular surface components. Consequently, dry eye is often classified as tear-deficient dry eye or evaporative dry eye. Tear–deficient dry eye is caused by a deficiency in aqueous tear secretion, as in Sjögren’s Syndrome (SS) and non- Sjögren’s Syndrome, and lacrimal gland dysfunction is the common feature of this condition. As a result, tear hyperosmolarity occurs because of the loss of the aqueous tear component.
Evaporative dry eye is caused by excessive evaporation. Evaporative dry eye is more common than tear-deficient dry eye, and as such is the most common form of dry eye. The main cause of this type of dry eye is believed to be meibomian gland dysfunction, but can also be seen with blepharitis, ocular mucin deficiency, blink disorders, disorders of the lid aperture, ocular surface disorders, and other tear film disorders, such as contact lens induced dry eye. Evaporative dry eye causes are either intrinsic, since they are due to intrinsic disease affecting the lid such as meibomian gland dysfunction, low blink rate or lid disorders; or extrinsic and are due to the changes in the ocular surface (including vitamin A deficiency, chronically applied topical anaesthetics and preservatives), contact lens wear, and ocular surface disease (allergic eye disease). The boundary between the intrinsic and extrinsic categories is not clear, but intrinsic causes are more common for evaporative dry eye.

Dry eye has different subjective symptoms, but most are similar to a foreign-body sensation, irritation, burning, blurring of vision, itching, tired eyelids, and photophobia. Closing the eyes can reduce some of these symptoms. Dry eye is usually associated with variable reported symptoms which can be identified by a defined list of questions, and that can result in straightforward comparisons between patients and visits.

The diagnosis of dry eye cannot be made by symptoms alone, because some of these symptoms can be caused by other conditions, and there is no single test of tear function in the clinic that can give a definitive diagnosis of dry eye. Current tests are numerous and vary widely in their specificity and sensitivity. Several tests are often used by combining the assessment of tear film stability by measurement of tear film break-up
time (BUT) or using the Tearscope (Keeler Ltd, Windsor, UK); the assessment of corneal desiccation using staining agents such as rose Bengal or fluorescein; the assessment of tear volume using the Schirmer and phenol red thread tests along with measurement of tear meniscus height.

The poor correlation that has been reported between the clinical signs and symptoms of dry eye \(^{42}\) means that there is often a lack of confidence in the diagnosis of dry eye, which may have an effect on the appropriate treatment being selected. This probably is a consequence of the limited number of valid tests available to clinicians for dry eye. It is possible that tear ferning may help with this, since symptoms may be related to tear osmolarity \(^{36}\), which tear ferning can describe. Furthermore, the prevalence of dry eye will increase as the proportion of people over 60 years of age grows; it is well recognised that dry eye is not a trivial complaint and needs to be solved.\(^{32}\)

Investigation of the tear film is challenging due to its small volumes available for analysis, and its transparent and dynamic nature. Furthermore, the unstable nature of a tear sample over time makes it a challenge for both science researchers and clinicians to decode the irregular and inadequate tear film components.\(^{43}\)

Clinicians and scientists recognised that biochemical analysis of osmolarity and key components in a tear sample is the way forward, but the small volumes involved make biochemical analysis particularly challenging. The first commercial device to measure the tear film osmolarity in the clinical setting was only available recently, although its precision and reproducibility is still emerging (TearLab, TearLab Corporation, San Diego, California, USA). Other laboratory techniques can be used to evaluate some components of the tear film, such as mucins, but none of these techniques can be used under clinical conditions.\(^{44}\)
A simple, tear film test that is quick and inexpensive to perform, and can indicate the biochemical properties of the tear film would be very useful.

**SAMPLE FERNING**

When a sample of body fluid, such as the tear film or saliva, is dried on a glass microscope slide, a crystallisation pattern, in the form of a fern, is produced (Figure 1). This phenomenon can occur with many body fluids and follows a characteristic formation process. The crystallisation begins with the formation of a nucleus, which consists of a regularly arranged number of ions. The nucleus is formed by aggregation as result of supersaturation of dissolved ions due to solvent evaporation at the peripheral edges of the drop. Each nucleus has the ability to grow into a large crystal unit where more ions were involved. When the sample solute is able to diffuse into areas with a lower solute concentration area, normal crystals can form. The process requires a slow crystals growth rate, low solution viscosity and low impurity levels to permit free solute diffusion.

**Figure 1 here**

The absence of such conditions can lead to dendritic crystal growth. In this situation the stems grow longer and branched at regular intervals along the main stem; however the reason for this regularity is not understood. Dendritic growth can be promoted by increasing the evaporation rate, reducing atmospheric humidity, increasing the drying temperature, or presence of low concentration of impurities. Such factors speed the crystallisation process, although the process could be slow with solute high concentration.
The first tear crystallisation was reported by Fourcroy and Vauquelin in 1791.\textsuperscript{47} The crystallisation phenomenon remained unstudied until 1946, when was observed by Papanicolaou during studying cervical mucus.\textsuperscript{48} Since then, ferning patterns have been used in a number of medical areas such as obstetrics and gynaecology to correlate vaginal and cervical mucus with the menstrual cycle,\textsuperscript{49} in early pregnancy,\textsuperscript{50,51} to assess the cervical mucus properties\textsuperscript{52} and to study changes in the conformation in ferning patterns and crystals during the menstrual cycle.\textsuperscript{53,54} Also, ferning has been used to study oestrogen activity and ovulation,\textsuperscript{51,53,55,56} and to determine the occurrence of ovulation.\textsuperscript{57}

It has also been used to test saliva,\textsuperscript{58} to correlate salivary ferning and the fertile period,\textsuperscript{59} and to consider the observation of salivary ferning as a new technique for determining the fertile period,\textsuperscript{60} and using salivary ferning in ovulation detection in family planning.\textsuperscript{61} However, some work has suggested that salivary ferning is an unreliable method to establish a women’s fertile period,\textsuperscript{62} and not associated with ovulation.\textsuperscript{63} The validity of saliva ferning test has also been investigated in the diagnosis of dry mouth in primary or secondary Sjögren’s Syndrome\textsuperscript{64} and its diagnosis.\textsuperscript{65,66}

**TEAR FERNING TEST**

Since the pattern of the tear fern depends on the composition of the tear sample, tear ferning has been suggested as a simple test for tear film quality at a gross biochemical level. Different techniques, such as glass capillary tubes, spatula, glass rod, cellulose acetate filter rods and Schirmer papers, can be used to collect tears (about 5–20 µl) from the eye (Figure 2), and then the collected tears are transferred immediately to a small
centrifuge plastic tube (0.5 ml or less). A sample (1–2 µl) is then pipetted onto a clean microscope glass slide and allowed to dry for 7–10 minutes under normal room temperature (20–26 °C) and room humidity (rH ≤50%). The slide then can be observed under light or digital microscope with different magnification (X≥ 10).

Figure 2 here

Depending on the tear film composition, a variety of ferning patterns can be observed; healthy tear sample produce full dense ferning patterns (Figure 3A), while the phenomenon of ferning pattern is fragmented or absent in a dry eye sample (Figure 3B). Tabbara and Okumoto\textsuperscript{67} were the first to report the use of ferning as a qualitative test for ocular tear deficiency. Abnormal tear ferning was found in 91% of patients with various forms of acute conjunctivitis, and a significant absence of ferning was noted in patients with diffuse conjunctival cicatrisation. These results suggested that tear ferning could be used as a simple and inexpensive test to evaluate the ocular tear film clinically.

Figure 3 here

The exact nature of what determines the pattern seen is still not fully understood. Tabbara and Okumoto\textsuperscript{67} were the first to call tear ferning as the ‘ocular mucous ferning test’, then Rolando\textsuperscript{68} called it the ‘tear mucous ferning test’ when he presented his grading
scale. This terminology suggested a causal link between tear ferning pattern and the ocular mucins.

Pearce et al.\textsuperscript{69} investigated the spatial location of organic (sulphur-containing) molecules in the tear fern and proposed a hypothesis of how ferning occurs. When the tear drop was allowed to dry, water evaporation lead to an increase in the solutes concentration and proteins can no longer to stay in solution and deposited at the margin of the drop. As the evaporation continues, the salt concentration also increases and spontaneous fern-like crystal formations appear. As the macromolecules, like mucin and protein, have been deposited at the margin of the drop, their absence removes any impedance to fern crystal growth. They suggested that macromolecules such as mucins and proteins are not in the actual fern structure and so do not play a direct role in the formation of the ferns, and as a result, the term ‘tear fern’ is preferred instead of ‘mucous fern’.

Hyperosmolarity has also been reported to affect the ferning patterns produced by deteriorating and modifying the ferning pattern, and so the tear ferning test has been suggested as being useful in the detection of hyperosmolarity in the tear film.\textsuperscript{70} This suggests a direct role for electrolytes in ferning. Kogbe and colleagues described the ferning phenomenon and the fern branching pattern as being reliant on the electrolyte concentration, particularly the ratio of monovalent sodium and potassium ions to divalent calcium and magnesium ions, and the ratio of the ions to proteins.\textsuperscript{44} X-ray and electron microscopic scanning of the structural composition of tear ferns has shown that tear fern crystals are composed of sodium and potassium chloride, with proteinaceous material controlling crystallisation indirectly by coating crystal faces and blocking fern extension,
and so the ratio of salt to macromolecular species does appear to be important in the
determination of tear ferning.\textsuperscript{71}

The shift in the salt-to-macromolecule ratio is the most convincing theory and helps
to explain the reduction of ferning quality and quantity in dry eye, where osmolality is
raised and combined with a reduced concentration of proteins and mucins. In this situation,
the concentrations of the chemical species involved in crystallisation are critically altered,
making dendritic growth no longer favourable.\textsuperscript{45}

Many factors can promote rapid crystallisation and ferning, such as evaporation
rate, temperature, mucous content, impurities, surface drying area, and sample viscosity.
The slow drying of a tear sample under a microscope cover-slip leads to a network
crystallisation instead of a fern pattern.\textsuperscript{45} High humidity can modify and deteriorate the
ferning patterns of tear fluid from normal eyes, and so stable environmental conditions are
important for the tear ferning test. It has been found that high temperatures and low
relative humidities provided high quality ferning, and slow evaporation, low temperatures
and high humidities led to larger sized crystals. The normal and best quality ferning
patterns appear to be produced when relative humidity (rH) is not higher than 50\%, and the
temperature range is between 20–26 °C.\textsuperscript{72}

\hspace{1cm}\textbf{CLASSIFICATION OF TEAR FERNING}

The first classification of tear ferning patterns was developed by Rolando \textsuperscript{68} and it
relied on assessing the spacing between the branches in the ferns. In Type I (Figure 4), the
ferns are closely-packed and no spaces between the branches. In Type II, the ferns are
smaller and have gaps between the branches, in Type III the ferns are small and incompletely formed with rare or no branching and the gaps become larger and wider, and in Type IV the ferning phenomenon is absent. Types I and II are observed in samples from normal healthy subjects (82.7% of normal eyes), and Types III and IV are associated with abnormal tear films disorders (91.7% of eyes with keratoconjunctivitis sicca).68

**Figure 4 here**

Although the Rolando scale is the most often used, it was not originally introduced for this purpose so there remains no clearly defined protocol. The intra-observer and inter-observer repeatability of the Rolando grading system has been examined.73 An 85.4% intra-observer agreement and an inter-observer agreement of 92.1% for the first run and 94.3% for the second run were found. They concluded that the Rolando system was an easy and consistent method for TF pattern classification, although the study has some methodology limitation. One limitation was the use of slides that represented only a portion of the entire tear ferning sample because of using 20X magnification, and that may have produced more variability and affected the number of agreements.73

Recently, the reproducibility of the Rolando grading scales has been examined by five different examiners comparing the TF patterns of both normal and Sjögren’s Syndrome subjects and confirm its reproducibility.74 However, in the study the TF patterns were scored using only the standard 4 grading types, and the use of increments, which is known to improve the sensitivity of grading,75 was not applied. This limitation will restrict the range of grading; making it more likely that agreement would occur.
Norn\textsuperscript{76} suggested a different classification system that depended on the fern branching angle. Fern shaped crystals (F) appeared in two forms: right-angle ferning (FR) and acute-angle ferning (FA). The acute-angle branches (FA) were found to be the norm in dry eye.\textsuperscript{76} However, this classification method is less refined than the Rolando grading scale, and thus less suitable for screening samples on a large scale.\textsuperscript{77}

Vaikoussis et al.\textsuperscript{78} used a five-grading scale of tear ferning in Sjögren’s Syndrome patients that was almost identical to the Rolando scale; Types 1 and 2 were similar in containing fully-branched ferns, but the frequency of branching was less in Type 2 with some empty spaces. In Type 3 there were large spaces without ferning and the branches looked more like snow crystals than ferns. In Type 4 ferns could not be recognised, and in Type 5 only clusters of crystals were evident, without any organised form of ferning.

**THE APPLICATION OF TEAR FERNING**

To date, there has been a limited use of tear ferning in the investigation of altered ocular surface conditions. The tear ferning test has been applied in different studies for both aqueous deficient dry eye and evaporative dry eye cases.

In studies involving aqueous deficient dry eye, it was used alongside the Rolando grading system in the diagnosis of kerato-conjunctivitis sicca (KCS), and was found to have high sensitivity (94\%) and specificity (75\%), which is comparable with other tests commonly used to test Sjögren’s Syndrome.\textsuperscript{79} It has been described as a specific, sensitive and simple test to estimate KCS in Sjögren’s Syndrome patients,\textsuperscript{78} and to be able to evaluate xerophthalmia and xerostomia in Sjögren’s syndrome, and for any other cases that complain of mucous membrane dryness.\textsuperscript{58} Tear ferning produced better diagnosis of KCS
in a group of rheumatoid arthritis subjects (sensitivity 82.2%, specificity 92.5% and
prognostic value 86.6%) than other tests.\textsuperscript{41}

It has been found\textsuperscript{80} that tear ferning appears to be independent of single tear
proteins, but rather that it is correlated with the secreted aqueous volume as well as some
correlation with tear film stability.

Tear ferning has been found to be a suitable diagnostic test for the assessment of
cystic fibrosis in which cystic fibrosis patients are characterised by an abnormally high
electrolyte concentration in exocrine secretions.\textsuperscript{81} The results suggested that the increased
hyper-viscosity of tears and the appearance of abnormal ferning patterns were due to an
increased electrolyte concentration.\textsuperscript{81} However, it has also been suggested that tear ferning
is not useful as a diagnostic test of cystic fibrosis, but rather as an indicator of clinical
status.\textsuperscript{82} An alteration in the tear ferning pattern in Down’s syndrome subjects has also
been observed, and this abnormality gives some indication of the causes for anterior
segment infectious pathologies.\textsuperscript{83}

With regards to evaporative dry eye, the tear ferning test has also been
recommended as a clinical test for all prospective contact lens wearers because it is very
easy to perform and will exclude patients with poor quality tears.\textsuperscript{84} Examining patterns of
soft contact lens wearers showed that tear ferning has good sensitivity (78.4%) and
specificity (78.4%) for predicting contact lens tolerance in a clinical setting. Type I
indicated a good contact lens tolerance, while Types II, III and IV were associated with
tolerance problems.\textsuperscript{85} However, it has also been recently reported that the tear ferning test
showed a poor correlation with tear film stability tests and ocular comfort index scores in
contact lens wearers and non-lens wearers, and displayed specificity (86%) and sensitivity
(50%) for the discrimination of tear film samples between contact lens wearers and non-lens wearers.\textsuperscript{86}

The first systematic study of tear film function in pinguecula patients revealed significant abnormalities in tear film stability and tear ferning.\textsuperscript{84} An improvement in tear ferning and tear function has been observed after pterygium excision, implying a relationship between pterygium and evaporative dry eye.\textsuperscript{87}

The tear ferning test has also been used in a number of studies in combination with other tear film diagnostic tests. It has been used to assess the eye sensitivity to draught using 7 different tests,\textsuperscript{88} to investigate the effect of long-term, locally applied, ocular medications on ocular surface and tear film mucous layer\textsuperscript{89} and in the study of eye discomfort and air pollution.\textsuperscript{90} Also, it has been used to establish the correlation between tear film stability and the presence of conjunctival concretions,\textsuperscript{91} and in several other studies that are shown in Table 1.

\textbf{Table 1 here}

Beden et al.\textsuperscript{105} used the tear ferning test to assess the quality of tears in premature and full-term new-born babies, and concluded that new-borns secrete good quality tears, albeit with moderate quantity with the Schirmer’s test. The study of tear ferning in normal women during the menstrual cycle found no effect from the menstrual cycle on tear ferning patterns.\textsuperscript{106} However, it was also reported that tear ferning can indicate changes in tear quality in post-menopausal women (PMW) with dry eye symptoms, revealing the higher osmolality in dry eye PMW compared with non-dry eye PMW.\textsuperscript{107}
Although there are many references reporting on the use of the tear ferning test, the underlying mechanisms responsible for producing tear ferning and their interaction with dry eye sub-types are still poorly understood.

CONCLUSIONS

Current clinical tests for dry eye correlate poorly with symptoms. Therefore, the results from these established clinical tests often don’t relate to patient symptomology, and so have limited sensitivity and specificity in the diagnosis of dry eye disease. However, the diagnosis of dry eye cannot be made by symptoms alone, because some of these symptoms can be caused by other conditions, with the result that there exists no single test of tear function in the clinic that can give a definitive diagnosis of dry eye. Tear ferning offers a simple and inexpensive laboratory test that can be used to evaluate dry eye and has features that allow it to be used in the clinic setting. Also, it has good potential for better understanding the biochemical mechanisms involved in the various types of dry eye.

Whilst several papers have been published in this area, there remain crucial gaps in the necessary knowledge in order for this test to make the transition fully from laboratory to clinic. Tear electrolytes and macromolecules both have the potential to produce tear ferns, but determining the factors which play important roles in tear ferning, and the optimum tear composition to produce normal ferning patterns has not yet been fully identified. Also, the Rolando grading system for tear ferning patterns has no defined protocol, and it can be difficult to interpolate between grades. Norm’s grading scale was found to be unrefined and less suitable for screening samples on a large scale, and while the Vaikoussis grading scale has more grades, it possesses the same inherent flaws as the Rolando scale (no defined
protocol and no indication of linearity), and is also based on samples from an abnormal population. There are no research papers that have attempted to use tear ferning to differentiate type of dry eye and a study to investigate the ability of tear ferning to do this needs to be undertaken.

These limitations in the tear ferning test encourage both researchers and clinicians to look for ways to develop the test further and to establish the optimum features of such a test to make it useful for tear film evaluation. A combination of the tear ferning test with other tear film tests in the clinic may then provide a real evaluation of the tear film and dry eye, and may help in the treatment of dry eye.
REFERENCES


Figure Legends

Figure 1. Different ferning forms (10 x magnification), (a): thick crystal fern, (b): thin crystal fern.

Figure 2. Collection of tear sample from the outer canthus using a glass capillary tube.

Figure 3. Tear ferning pattern produced from healthy tear (A) and from dry eye (B).

Figure 4. Images of Rolando Grading Scale show progression in tear ferning pattern. Images are from a laboratory collection, but are matched to Rolando scale images.

Table 1. Summary table showing the use of tear ferning as a diagnostic test.
Table 1. The use of tear ferning as a diagnostic test

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<td>98</td>
<td>Using hypotonic 0.4% hyaluronic acid drops to study the improvement of the ocular surface in KCS.</td>
<td>Treatment with 0.4% hyaluronate can improve the epithelial condition of the ocular surface.</td>
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<td></td>
<td>In primary open angle glaucoma subjects with and without history of allergic conjunctivitis to evaluate the effect of latanoprost and timolol on the ocular surface.</td>
<td>Latanoprost treatment induces ocular surface changes.</td>
<td></td>
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<tr>
<td>99</td>
<td>To investigate the changes in the tear film and conjunctiva after diet supplementation of Vitamins C and E in non-insulin dependent diabetes mellitus.</td>
<td>Ocular surface milieu probably improved with diet supplementation of anti-oxidant Vitamins C and E.</td>
<td></td>
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<tr>
<td>100</td>
<td>To analyse the ocular surface in patients affected with rheumatic diseases.</td>
<td>Results suggest that tear ferning can enlarge the spectrum of ocular surface analysis.</td>
<td></td>
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<tr>
<td>101</td>
<td>To establish the correlation between tear film stability and the presence of conjunctival concretions.</td>
<td>Tear film deficiency may be present in patient with conjunctival concretions; ferning test of 5 patients showed normal patterns in all of them.</td>
<td></td>
</tr>
<tr>
<td>91</td>
<td>To know if dry eye symptoms and ocular surface parameters change in women subjects with and without dry eye during the menstrual cycle.</td>
<td>Clinician should take into account the cyclic variation during examination of dry eye.</td>
<td></td>
</tr>
<tr>
<td>102</td>
<td>In the evaluation of the diagnostic performance of tear film tests in primary Sjögren’s Syndrome (SS-I) subjects.</td>
<td>Results confirm the scarce reliability of Schirmer test I and BUT, and recommended vital dye staining as the best test in SS-I differential diagnosis.</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td>To investigate tear film stability, volume, bulbar hyperaemia, tear ferning and the subject’s subjective symptoms pre- and post-sleep.</td>
<td>Eye dryness and discomfort symptoms are worse following overnight eye closure.</td>
<td></td>
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<td>104</td>
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Figure 1. Different ferning forms (10x magnification);

(a): thick crystal fern, (b): thin crystal fern.
Figure 2. Collection of tear sample from the outer canthus using a glass capillary tube.
Figure 3. Tear ferning pattern produced from healthy tear (A) and from dry eye (B).
Figure 4: Images of Rolando Grading Scale show progression in tear ferning pattern. Images are from a laboratory collection, but are matched to Rolando scale images.