REVIEW

Tear instability importance, mechanisms, validity and reliability of assessment

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Dry eye; Tear instability; Break up time

Abstract
Purpose: To examine the factors which contribute to tear stability and the validity and reliability of methods used for assessing tear break up time which is a core part of an examination of tear stability in dry eye patients.
Methods: A review of publications which are relevant to tear stability and its assessment.
Results: Tear break up time may be more invasive than intended if difficulty avoiding blinking during assessment results in reflex tearing. Notwithstanding control of instilled volume and concentration of fluorescein, on-eye dilution is highly variable according to resident tear volume. Blinking to evenly distribute fluorescein may improve tear and lipid layer thickness so habitual tear function is not assessed. Emphasis on a role for Meibomian gland dysfunction as a cause of tear instability may be appropriate in many cases but ignores the roles for other sources of tear lipid and other non-lipid contributions to tear instability such as aqueous or mucus deficiency, desiccated epitheliopathy or anomalous blinking. Objective less-invasive methods eliminate problems of inter-observer variability and can reliably ‘maintain vigilance’ over wide areas of the tear layer. However less-invasive results to date include mean tear break up findings which are both shorter and longer than expected for normal controls.
Conclusions: Fluorescein tear break up time assessments cannot be standardised and less-invasive methods are not yet standardised. Objective less-invasive and subjective fluorescein break up time tests do not appear to be measuring the same tear phenomena although both should be performed before other invasive procedures.

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Assessment of tear break up time (TBUT) is a core measure of tear stability and its measurement is a major cornerstone of clinical tests for dry eye as an indication of the rate of tear loss by evaporation. This measurement has the potential to capture the combined contributions of lipid, mucin and aqueous deficiencies to tear instability for example. This review examines the mechanisms and factors which determine tear stability and instability as well as the variables involved in their measurement because the methods used to achieve reliable assessments and to establish appropriate diagnostic criteria depend on the degree of understanding and control over those variables. PubMed searches using the terms ‘tear break up time tests’, ‘tear instability’, and ‘tear evaporation’ yielded 382, 2306 and 313 potentially relevant publications respectively. Selections from these lists were made to examine the evidence which appears to be the most relevant for examining the mechanisms and variables which determine tear stability as well as to assessing the validity and reliability of measuring TBUT as an indication of tear stability.

The potential significance of evaporation in aqueous deficient dry eye (ADDE) when tear stability is normal range

Although excessive evaporation is a core factor in cases of evaporative dry eye (EDE) even normal evaporation rates can be important contributors to the symptoms which develop in ADDE. Notwithstanding normal lipid and mucin functions in some cases, very thin tear layers in ADDE eyes are susceptible to TBU and associated symptoms due to tear loss which occurs with normal rates of evaporation. This relationship is indicated by the finding that, compared to normal controls with a mean fluorescein TBUT (FTBUT) of 7.1 s, mean FTBUT for patients with ADDE was 2.1 s. Similarly, mean non-invasive TBUT (NITBUT) was found to be 3.3 s for non-Sjogren’s Syndrome ADDE subjects compared to 6.6 s for subjects with MGD and normal tear layer thickness. Consequently, ADDE may include symptoms with an evaporative basis which are similar to those which develop in EDE which occurs without an ADDE component. The susceptibil-
ity to evaporation-based symptoms is even greater in ADDE when a thin tear layer is unstable due to lipid and/or mucin deficiency.

**Provocative conditions for evaporation**

Evaporation is increased by air movement, low humidity, high temperature and by the duration of exposure to ambient conditions as determined by blink frequency (interblink interval (IBI)) and blink completeness. Although evaporative thinning is increased for the entire exposed ocular surface by a low blink rate, incomplete blinks approximately double the duration of exposure to evaporation for the overexposed inferior ocular surface.7 That the use of subjective and objective NITBUT methods in normal subjects detected tear instability most commonly in the inferior cornea (45.7%) compared to the superior cornea (5.0%) is consistent with the inferior corneal surface being more exposed to evaporation by incomplete blinks.8 Accordingly, increased corneal staining of the inferior cornea was observed in patients with greater rates of incomplete blinking9 and the relative proportion of incomplete blinks was much higher in patients with inferior punctate keratopathy.10 Inferior corneal staining was found to be the most consistent diagnostic measure providing some validation for its use as a primary endpoint in clinical trials of dry eye.11 Inferior corneal staining showed a small but significant diurnal increase for both normal and dry eye subjects.11 The positive correlation between diurnal changes in inferior corneal fluorescein staining and mean daily IBI in a normal group suggests a possible relationship between cumulative environmental effects and staining under conditions of normal blink dynamics.10 As discussed below, inferior corneal epitheliopathy may influence tear stability and the development of TBU in affected areas during assessment of TBUT.

**Lipid deficiency, tear instability and evaporation**

Evaporation of tears occurs during an IBI, even when tears are healthy,12-14 and is associated with increased osmolarity. The precorneal tear film undergoes a formation (build up) phase immediately after a blink.15 A subsequent relatively stable inter-blink phase can be destabilised by break up in subjects with dry eye or in normal eyes when the inter-blink phase is abnormally extended.15 When blink rate and completeness are at least adequate, the healthy tear is reformed to normal thickness by blinking before any TBU and before evaporative thinning and associated increased osmolarity reach levels which stimulate symptoms. TBU may be the consequence of Meibomian gland dysfunction (MGD) and associated regional variations in lipid layer protection from evaporation with greater thinning in lipid deficient areas.4 Apart from MGD, there may be other contributions to tear instability. For example, most of the tear lipids are produced by the MG but it has been shown that the MG could not be their only source.17 Lipidomic analysis of human tear lipid indicated the possibility of a lacrimal gland origin of some tear lipids.18 Butovich and coauthors proposed that conjunctival and corneal epithelial cells could produce some tear polar lipids.19 Lipophilic substances from the lower lid surface are able to reach the inferior tear meniscus supracutaneously and mix with the tear film layer.20 Sebum is produced from the glands of Zeiss and Moll and, as there is no boundary between sebum and meibum from the MG, some mixing of them could occur.20 In addition, tear lipids may become contaminated by skin lipids or by unnatural sources such as sun tan lotions, moisturisers and other cosmetics.21 The evaporating inhibiting function of the lipid layer appears to potentially be influenced by lipid contributions from any of these various sources.

**Mucin deficiency, tear instability and evaporation**

Emphasis on MGD contributions to tear deficiency and a lack of satisfactory methods for clinical assessment of mucin functions22 may allow an underappreciation of mucin contributions to tear stability to develop. The glycolalxy has previously been referred to as the mucus layer which, in the healthy eye prevents the epithelial surface from dewetting.23 Consistent with the then current understanding of tear function the FTBUT test devised by Norn 196924 was originally intended to be used as a measure of mucin deficiency. This model was supported by the finding that shorter TBUTs correlate with reduced goblet cell density.15 Apart from helping to maintain tear stability, mucins help with the lubrication of lid movements26 and protect against lid wiper epitheliopathy.27 Short TBUT findings may be partly a consequence of qualitative and/or quantitative mucin dysfunction. Goblet cell density (the prime source of secretory mucins) can be determined by conjunctival impression cytology which is a relatively cumbersome and time consuming procedure.28 The ocular surface epithelium is a secondary source producing transmembrane mucins such as MUC1, MUC2 and MUC4.29 The lacrimal gland produces MUC7.29 An in vitro study found that the application of a mucin secretagogue induced the expression of mucin, increased the number of mucin-secreting cells and thickened the thin film layer generated by mucin and aqueous secretion.20 That mucin contributes to tear stability is illustrated by the finding that in human dry eye subjects 3% diquafosol was found to increase mucin concentration in tears as well as to increase TBUT.31 A scanning laser confocal microscopy 3D image analysis of conjunctival impression cytology findings was used to determine goblet cell density and goblet cell layer thickness.32 This technique identifies goblet cells which are not secreting mucins.32 Reduced tear stability in patients with symptoms of dry eye was found to be primarily due to decreased mucin production compared to control subjects without dry eye.33 A tear-fenring test may be useful in evaluating levels of mucin activity but lack of a standardised examination protocol and a reliable grading scheme limit its clinical application.26 However, mucin dysfunction may be suspected when TBUT is short as well as when indicators of reduced mucin-related lubrication of blink movements over the ocular surface such as lid wiper epitheliopathy and lid parallel conjunctival folding are present.26 Epitheliopathy over the pupil is associated with increased higher order aberrations and backward light scattering25 and any abnormal distribution of mucin over a desiccated area of
epitheliopathy could also contribute to tear instability and shorter TBUT.

Tear thinning, break up, hyperosmolarity and symptoms

The thinning of the tear film and TBU during IBIs are complex processes which, apart from evaporation, and mucin deficiency-related dewetting\(^1\) can involve divergent tangential (pressure-gradient) flow from an area of TBU.\(^2\) Divergent tangential flow may be driven or drawn from a TBU area by the physical force of surface tension gradients.\(^3\) This form of TBU may be aided by lipid contamination of the mucin layer in that area, and the associated hydrophobic nature of the exposed epithelial surface.\(^4\) Normally the presence of the hydrophilic glycolycan on the healthy ocular surface prevents the tear film from dewetting.\(^5\) Elevated rates of exfoliation of aged cells may cause the tear layer to be thinner in some areas.\(^6\) Tear thinning may also occur in areas of epitheliopathy due to desiccation, to the extent that such areas involve increased cell exfoliation and associated cell elevation. The inclusion of ocular surface wettability in a model of tear dynamics involved break up reaching a nonzero equilibrium thickness\(^7\) indicating that a break up area need not be dry in the sense of being devoid of aqueous as discussed further below. Tear osmolarity increases according to the degree of evaporation and associated reductions in tear volume. Evaporation may not occur evenly over the ocular surface when tears are unstable and areas of significantly greater evaporation and TBU could develop and elevate osmolarity in those areas. Hyperosmolarity of tears due to evaporation and/or break up has been estimated to reach 800–900 mOsm/L.\(^8\) Short TBU was found to be a useful surrogate marker for tear hyperosmolarity\(^9\) which is also a core mechanism for dry eye symptoms.\(^1\) Irritation and reflex tears which increase tear layer thickness may confound TBU evaluation.

Evaporation, tear break up and vision deterioration

Visual disturbance can result from non-uniform tear film thinning as well as exposure of a rough epithelial surface which is associated with light scatter and gross wavefront aberrations occurring within areas of TBU.\(^10\) Within 3–4s after a blink, significant loss of acuity can be experienced.\(^11\) Such findings are consistent with tear instability and/or anterior tear surface irregularity which results in less than optimum refraction. Increased blink rates help to improve vision in these cases but may also explain symptoms of tired eyes.\(^12\) For example, eye fatigue can occur when patients with dry eye struggle to see and need to blink more frequently to clear their vision.\(^13\) For contact lens wearers, measures of tear quality and retinal image quality are also associated with the decline in vision which occurs with TBU.\(^14\)

Fluorescein instillation, dilution and tear break up time

Measurement of FTBUT could be the most commonly used evaluation of tear function\(^3\) although methods for its application and conditions for examination vary widely. Standardisation is intended by control of the volume and concentration of sodium fluorescein (NaFl) instilled.\(^15\) For an ADDE with tear volume of 3 \(\mu\)L, an instillation of 1 \(\mu\)L of NaFl represents 25% of the total. Assuming a normal range tear volume of 7 \(\mu\)L,\(^16\) instillation of 1 \(\mu\)L of NaFl represents only 12.5% of the total volume and dilution of NaFl dilution is doubled compared to the ADDE example. Mean tear thickness in ADDE was found to be 2.0 ± 1.5 \(\mu\)m indicating that for advanced cases, tear films can be ultrathin and dilution of instilled NaFl very limited by comparison with eyes having normal range tear volumes.\(^17,18\) Spiking of hyperosmolarity as tears evaporate and break up was found to have the potential to generate inflammatory responses which have the associated potential to stimulate sensory neurons and irritation\(^19\) in both ADDE and EDE. Cooling of tears (latent heat of vaporisation) during evaporation may activate dryness detecting sensitised low-threshold C-mechanoreceptors.\(^20\) This type of cooling stimulus may also contribute to evaporation-related symptoms\(^21\) in both EDE and ADDE. Reflex tears may be responses to irritation associated with evaporation-related hyperosmolarity and/or tear cooling, especially in a break up area\(^22\) which develops during TBU assessment. By following instructions to not blink during assessment patients may experience irritation in some form which stimulates reflex tears. An instruction “to only blink if your eyes become irritated” is unlikely to prevent reflex tearing. A patient who is compelled to blink appears likely to have experienced irritation and produced reflex tears which may also be stimulated by irritation in the contralateral eye.\(^23\) NaFl dilution is greater according to the degree that reflex tears are stimulated. That reflex tearing occurs during assessment may become evident by reassessment of the lipid layer interference pattern or tear meniscus height.\(^24\) In addition to NaFl dilution,\(^25\) reflex tears may confound test results by contributing to a departure from a patient’s normal tear structure. For example, instillation of a drop of saline was found to thin lipid layer interference patterns\(^26\) which appears to help explain how instillation of NaFl caused an increase in evaporation rate.\(^27\) At the dilute limit, the NaFl concentration is below the critical concentration and the intensity of the fluorescence from the tear film is proportional to its thickness.\(^28\) Evaporation during assessment may cause an area of diluted NaFl to be thinned sufficiently so that it no longer reaches the threshold for the detection of fluorescence. Thus dilution of NaFl may result in a darker area being judged to be a dry break up area when the tear layer of diluted NaFl is thin rather than absent.

Blinking after fluorescein instillation

That patients are required to voluntarily blink fully three or more times to ensure distribution of NaFl\(^1\) appears likely to also alter tear structure. The preocular tear film undergoes a formation (build up) phase immediately after a blink\(^9\) so that a thin tear film is thickened by blinking which evenly
distributes the tears over the ocular surface.\textsuperscript{51} Blink-related tear layer thickening appears likely to reduce susceptibility to significant evaporation. For example, Kojima and co-authors found that punctum plug insertion improved tear stability\textsuperscript{52} presumably in association with aqueous retention and a thicker tear layer. Blinking can also induce lipid secretion from MGS\textsuperscript{55,56} especially perhaps when greater force is involved. Instruction to blink gently following NaFL instillation can result in incomplete blinking and poor fluorescein distribution. Instructions to blink fully to avoid incomplete blinks and improve NaFL distribution may encourage unnatural voluntary blinks which involve greater force\textsuperscript{57} and the possibility of increased lipid secretion so that evaluation becomes further removed from any habitual tear dysfunction. Apart from lipid layer enhancement, mucin spreading and associated improved ocular surface wettability may also be associated with pre-assessment blinks needed to distribute instilled NaFl. Conversely, stretching and thinning of the tear film over a larger area due to widening of the palpebral aperture\textsuperscript{58} may be a consequence of an instruction and efforts to avoid blinking during an assessment, and could reduce BUT. Alternatively, narrowing of the palpebral aperture as may be prompted by irritation could thicken the tear layer. As discussed above, notwithstanding the instillation of a controlled volume of NaFl, there will be less dilution and a greater concentration of fluorescein on an ADDE. High localised concentrations of NaFl and quenching of fluorescein can create the appearance of a dry dark break up area.\textsuperscript{39} Evaporation which occurs while waiting for any break up to develop increases NaFl concentration. Again, any associated fluorescein quenching reduces fluorescent intensity and the darkness in such an area may be interpreted incorrectly as a dry break up area.\textsuperscript{60-62} Depending on the quantity and concentration of NaFl instilled and the dilutitive influence of the resident tear volume, pre-assessment blinks and/or reflex tearing, subsequent findings may not be representative of habitual tear function or dysfunction.

Non-invasive tests of tear break up time

A non-invasive technique for assessing tear film does not involve instillation of any substance, has no physical contact with the eye or adnexa and does not require voluntary blinking.\textsuperscript{63} For example, NITBUT assessments can examine for perturbations of grid or plaido disc images reflected by the tear layer anterior surface.\textsuperscript{49} Slit lamp observations of a grid pattern image reflected from the cornea found that 80% of NITBUTs for an unselected sample of subjects were >30s.\textsuperscript{49} Such longer TBU findings for subjective observations may be a consequence of failure to detect the earliest evidence of TBU. Another subjective NITBUT method involves assessment using a grid pattern image-based Tearscope\textsuperscript{®} type of instrument independently or as an attachment to a slit lamp.\textsuperscript{49} Nichols and co-authors found considerable inter-examiner variability with subjective NITBUT Tearscope\textsuperscript{®} assessments.\textsuperscript{58} More recently a variety of objective NITBUT assessment methods have been developed.\textsuperscript{41,51,65-70} However, sometimes mean NITBUT findings appear to be too short for normal control subjects\textsuperscript{66,67,70} and sometimes they appear to be too long.\textsuperscript{41} These variations may be a function of different forms of raw measurement data as well as differences in the software used to convert raw data into TBU. Further development of software may reduce the extent of these apparent anomalies\textsuperscript{66} but at this time the findings from different forms of FTBUT and NITBUT assessment are not interchangeable.

Findings of zero seconds for NITBUT\textsuperscript{71} may result from the detection of tear layer surface irregularity rather than being due to evaporative TBU. For example, abnormal tear layer quantities of foreign matter, cellular tear debris, mucoid corneal filaments and/or lipid clumps\textsuperscript{53} could cause irregularity in the anterior tear surface layer and result in NITBUT findings of zero seconds, especially in advanced ADDE when tear layers are very thin. In a dry eye group, for example, the most commonly observed lipid layer observation was an abnormal-colour fringe interference pattern.\textsuperscript{72} This type of tear layer features clumps of lipid floating in areas of exposed aqueous and is associated with poor tear stability.\textsuperscript{73} Tear rupture can also appear immediately following a blink when the mucous layer in the area of rupture has been contaminated by lipid which results in a fixed dry spot.\textsuperscript{49} This possibility might be more likely if skin lipids befoul the lipid layer such as may occur in blepharitis for example.\textsuperscript{69} Similarly, oil-based cosmetic products or sun-tan lotions can contaminate the tear lipid and mucous layers\textsuperscript{74} and reduce TBU. Very low TBU findings may also be a consequence of natural ocular microfluctuations in eye position which can be detected when tear instability is derived from topographic data analysis such as surface regularity and asymmetry indices.\textsuperscript{73,74} These microfluctuations are the result of lateral fixation shifts and cyclorotations which contribute to increased variance of measurements.\textsuperscript{74} Iskander and co-authors have developed a non-invasive measure of tear stability termed the tear film surface quality breakup time which has been derived from dynamic area high speed Placido disc Medmont\textsuperscript{®} videokeratography to compensate for microfluctuations.\textsuperscript{75} Zero or very low NITBUT findings may prompt a patient being given an instruction to blink several times to try and clear excess tear debris but, as described above in relation to the distribution of NaFl, such an instruction may reduce the chance of an assessment that represents a normal interblink condition.

The Tear Stability Analysis System\textsuperscript{®} (TSAS) was designed for the Tomey Topographic Modelling System (TMS-2N, Tomey Corporation) and uses a topographic modelling method of calculating TBU values based on changes in the differences in brightness of individual measurement points on mire rings.\textsuperscript{73} The TSAS break up time highest value for normal subjects was 6s compared to 1.2s for dry eye subjects.\textsuperscript{73} Using a different version of the TSAS the mean BUT for normal controls was 4.91 s.\textsuperscript{59} These TSAS findings appear to be ultrasonometric compared to cut-offs of 5 or 10 s which have been recommended for FTBUT assessments.\textsuperscript{76} An Oculus Keratograph K5 (Wetzlar, Germany) equipped with modified TF-scan software was used in a dark room to examine NITBUT in 44 dry eye and 41 normal subjects with results compared to FTBUT determined by instillation of 2 µL of a preservative free 1% NaFl solution.\textsuperscript{70} For all subjects, mean NITBUT was 3.2 s and significantly shorter than mean FTBUT which was 5.2 s.\textsuperscript{70} Apparently the TSAS and Keratograph K5 are capable of detecting tear instability or irregularity which would not ordinarily be detected during subjective FTBUT assessment. These findings raise the question of what level
and type of instability is clinically significant? A Placido disc videokeratographer (Medmont 300®), Medmont International Pty Ltd, Victoria, Australia) was used to examine “tear film surface quality break up time” (NITBUT) for 28 DES subjects and 17 healthy controls. In contrast with the TSAS and Keratograph findings, the mean NITBUT was 13.4 s for DES subjects and 21.3 s for controls. The longer findings may be a consequence of raw data analysis which corrects for microfluctuations in eye position.73,74

**Discussion**

Measuring and understanding tear layer instability may be progressed by a better understanding of tear layer stability.75 Ideally, TIBUT assessments capture a valid estimate of tear instability which is consistent with symptoms and diagnosis of a dry eye disease (DED). This cannot always be the case when symptoms which are reported by DES patients with unstable tears are caused by or are associated with allergy, anterior blepharitis or neurogenic mechanisms for example. Emphasis on a role for MGD in causing tear instability is appropriate but the roles for other sources of lipid deficiency as well as non-lipid contributions to tear instability from aqueous deficiency, mucus deficiency, blinking anomalies and areas of desiccated epitheliopathy may be relevant for example. Consequently, MG function assessment alone cannot always be used as an indication of tear instability. There are several uncontrolled variables involved in FTIBUT assessments such as restoration of tear and lipid layer thickness as well as improved mucin distribution all of which could result from pre-assessment voluntary blinking. These findings may not be representative of habitual tear dysfunction.

A single drop of either saline or an artificial tear solution was found to increase tear evaporation rates in healthy control subjects without histories of dry eye.76 FTIBUT assessment has been criticised as inaccurate and not reproducible due lack of standardisation for volume and concentration of NaFl instilled.62 On-eye NaFl dilution also varies widely according to resident tear volumes as well as in response to any reflex tearing associated with evaporation and increased osmolality which occurs during assessment. Apart from variable NaFl volume and concentration, the degree of magnification and/or the use of a Kodak Wratten 12 yellow filter used to detect TBU are not necessarily standardised. High magnification facilitates the detection of TBU but reduces the area which can be scanned effectively. Because methods to measure TBU vary widely, the cut-off for DES diagnosis varies accordingly.76 That symptoms and signs of DES are typically worse for Asians compared to Caucasians77 raises the possibility that criteria for diagnosis may need to be varied according to ethnic differences. For example, NITBUT for healthy tears was found to be shorter in Malays compared to Western populations.86 Methods of measuring NITBUT avoid the potential for FTIBUT assessment inconsistencies and eliminate inter-observer variability found with subjective methods.65 That diagnostic criteria for FTIBUT and NITBUT are different has a long88 as well as current history.63 suggesting that NITBUT and FTIBUT may not be measuring the same tear phenomena. For example, sometimes NITBUT may detect an area of irregularity of the tear anterior surface associated with tear debris rather than an evaporative break up area. The question of how many assessments should be made during the same examination appears to be far from settled. Best and co-authors found that second Keratograph NITBUT was an average of 1.64 ± 0.03 s less than the first (p < 0.01)68 which may be a consequence of the method of examination being more invasive than intended. For example, reflex tear production might alter NITBUT. Chen and coauthors reported that the mean for multiple NITBUT measurements was longer than for the first reading (12.3 s vs. 9.7 s)69 which may be a consequence of blinking in between assessments so that tear volume and stability improve during an assessment sequence. For both subjective and objective methods TIBUT is evaluated under forced-stare conditions subsequent to “do not blink” instructions and reflex tearing occurs so that NaFl dilution is increased accordingly.62 Methods which are preceded by instructions to blink normally three times65 evaluate a tear layer which has been restored and under these conditions NITBUT could be more accurately described as less-invasive TBUT. The pursuit of more valid and reliable measures of TBUT is more than justified because tear instability is a core feature of DED71-73 but also because, apart from lipid, mucin and aqueous deficiencies, TBUT can be a surrogate marker for tear osmolality, which is another core feature of DE.1

**Conflicts of interest**

The author has no conflicts of interest to declare.

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