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Minimising instilled volume reduces the impact of fluorescein on clinical measurements of tear film stability

Jennifer K. Mooi[a,b], Michael T.M. Wang[a], Joevy Lim[a], Andreas Müller[a,c], Jennifer P. Craig[a,*]

[a] Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, New Zealand
[b] Warringal Private Hospital, Heidelberg, VIC, Australia
[c] Centre for Eye Research Australia (CERA), Melbourne, VIC, Australia

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ABSTRACT

Purpose: To compare clinical tear film break-up time measurements obtained non-invasively, with those measured following minimal and conventional volumes of fluorescein instillation.
Methods: Forty-one subjects (20 male, 21 female, mean ± SD age 34 ± 11 years), with or without dry eye, participated in a prospective cross-over study. Tear film break-up time was measured by the Tearscope Plus™ with fine grid insert. Measurements were made in triplicate, with no fluorescein instillation (NIBUT), then following application of a minimal volume of 1 μl fluorescein from the Dry Eye Test™ (mTBUT), and finally with 15–30 μl of fluid instilled via a conventional fluorescein strip (TBUT). A fifteen-minute interval between each set of measurements minimised the risk of residual contamination effects.
Results: All three techniques displayed statistically significant pairwise correlation (all $p < 0.001$). TBUT values were significantly shorter than both NIBUT (geometric mean 8.6 s versus 10.9s, $p = 0.03$) and mTBUT (geometric mean 8.6 s versus 10.6s, $p = 0.03$), and demonstrated narrower spread (both $p < 0.05$). No significant differences were detected between NIBUT and mTBUT (all $p > 0.05$).
Conclusions: Tear film break-up time values measured with conventional fluorescein instillation were shortened, while minimal fluorescein instillation and non-invasive methods produced comparable readings. This suggests that minimising instilled volumes can reduce the impact of fluorescein on clinical measurements of tear film stability.

1. Introduction

Tear film stability assessment is an essential component of the diagnosis and evaluation of dry eye [1,2]. It can be measured by the tear film break-up time, defined as the interval between a blink and the first occurrence of dry spots on the cornea [3]. Tear film stability is generally reduced irrespective of the aetiology of dry eye [4].

Tear film stability is commonly measured in the clinical setting with the fluorescein break-up time test [5–7]. Instilled aqueous fluorescein sodium enhances tear film visibility under blue light, thus simplifying the measurement [8]. However, conventional saline-wetted fluorescein-impregnated strips deliver a variable volume of fluid, typically around 15–30 μl, and in excess of the natural tear volume, which can destabilise the tear film [9,10]. Shaking off excess fluid from wetted fluorescein strips prior to application can reduce the volume instilled and improve the resulting visualisation [8].

Non-invasive measurement techniques using reflected mires to facilitate observation of tear break-up are considered to be superior, by avoiding the presumed destabilising action of fluorescein. Non-invasive measurements have previously been reported to be significantly greater than conventional fluorescein break-up times measured from the same tear film [9,10]. However, the conventional fluorescein test remains widely used clinically [5–7], largely on account of its relative convenience of measurement, ease of interpretation and low cost, relative to that perceived to exist with non-invasive instrumentation.

The Dry Eye Test™ (Amcon Laboratories, St Louis, MO, USA) provides a modified form of tear stability assessment, using a proprietary strip design that delivers consistently smaller volumes (1 μl) of instilled fluorescein than conventional strips. The Dry Eye Test has previously been reported to have greater measurement reliability and precision than conventional fluorescein strips [11], however its comparability with non-invasive techniques is not currently known.

* Corresponding author at: Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, Private Bag 92019, Auckland, 1142, New Zealand.
E-mail address: jp.craig@auckland.ac.nz (J.P. Craig).

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This crossover study assessed the invasiveness of Dry Eye Test relative to a non-invasive technique and the conventional fluorescein test, in the clinical setting, and investigates whether minimising the instillation volume of aqueous fluorescein can diminish the level of tear film disruption.

2. Methods

2.1. Subjects

The prospective crossover study followed the tenets of the Declaration of Helsinki and was approved by the institutional Human Participants Ethics Committee. Participants were required to be between 18 and 40 years of age, non-contact lens users, with no history of major systemic or ocular disease (other than dry eye), no previous ocular surgery, and no topical or systemic medications affecting the eye. Eligible participants were enrolled after providing written informed consent.

A total of 41 eligible participants (20 male, 21 female), with a mean ± SD age of 34 ± 11 years, were recruited. This exceeded the sample size requirement for the desired study power. Power calculations showed that a minimum of 33 participants was required, to detect a clinically significant difference of 5 s, with 80% power ($\beta = 0.2$), at a two-sided statistical significance level of 5% ($\alpha = 0.05$). The SD of normal values was estimated to be at 7 s [12]. Sample size estimates were determined using a uniform non-parametric adjustment, with PASS 2002 (NCSS Statistical Software LLC, Utah, USA).

2.2. Measurements

All subjects were assessed in the same location, with a mean ± SD room temperature of 22.0 ± 0.5 °C and a mean ± SD relative humidity of 49.0 ± 6.0%. Measurements were conducted by a trained research technician and therapeutically qualified optometrist.

Tear film stability measurements were performed on the right eye of each participant, using the Tearscope Plus™ (Keeler Ltd, UK), with fine grid insert in place, in all cases [13]. The tear film break-up time was measured in the same order for each subject under three different clincial scenarios: non-invasively with no fluorescein instillation (NIBUT); following application of a minimal volume of approximately 1 μl fluorescein from the Dry Eye Test (mTBUT); and following instillation of fluorescein in 1 drop (15–30 μl) of saline from a conventional (Haag-Streit, UK) fluorescein strip (TBUT), each applied according to the respective manufacturer's instructions, with excess fluid shaken off prior to application. The fluorescein was applied to the superior-temporal bulbar conjunctiva while participants were instructed to look infero-nasally.

Participants were instructed to blink naturally for 1 min to facilitate even distribution of fluorescein over the ocular surface. Subjects were then instructed to refrain from blinking while the examiner observed the reflected grid pattern. The tear film break-up time was recorded as the time between the blink and the first sign of distortion in the grid pattern. A mean of three measurements was recorded for each technique. Fifteen minutes were allowed to elapse between each set of measurements to minimise the risk of residual contamination effects.

2.3. Statistical analysis

Statistical analyses were performed using Graph Pad Prism version 6.02 (http://www.graphpad.com) and IBM SPSS Statistics for Windows version 19.0. The distributions of tear film stability measurements were assessed using the D'Agostino-Pearson omnibus normality test. Consistent with previous reports, tear film stability measurements were non-normally distributed [14–16], and thus both the geometric mean and the median are presented. The non-normally distributed measurements were then logarithmically transformed before further analysis. Comparisons of means were performed using repeated measures analysis of variance (ANOVA). Post-hoc analyses for pairwise comparisons were conducted using multiplicity adjusted Tukey tests. Comparisons for variances were undertaken using the *F*-test. For each pairwise comparison, the intra-class correlation co-efficient (ICC) was calculated, and Bland-Altman analysis performed [17]. All tests were two-tailed and $p < 0.05$ was considered significant.

3. Results

Tear film stability data from all three techniques were positively skewed, and failed normality testing (all $p < 0.001$). The distributions of tear film break-up time measurements obtained from the three techniques are illustrated in Fig. 1 and Table 1, and pairwise analyses are shown in Table 2.

Following logarithmic transformation, the distribution of values from each technique did not differ significantly from normal distributions (all $p > 0.05$). Significant differences were detected between the measurements obtained from the three techniques ($p = 0.01$). Post-hoc analysis showed that both NIBUT and mTBUT measurements were significantly longer than TBUT (all $p < 0.05$), while there were no significant differences between NIBUT and mTBUT ($p = 0.84$). Both NIBUT and mTBUT measurements demonstrated a larger spread than those of TBUT (all $p < 0.05$), although NIBUT and mTBUT did not differ significantly ($p = 0.87$).

In all three pairwise analyses, the stability techniques were found to be significantly correlated (all $p < 0.001$). The intraclass correlation coefficient was greater for NIBUT versus mTBUT, than for mTBUT versus TBUT, and NIBUT versus mTBUT. Bland-Altman analysis also demonstrated a smaller bias and narrower limits of agreements for NIBUT versus mTBUT (Fig. 2), than the other two pairwise comparisons (Figs. 3 and 4). Adjusting the Bland-Altman mean biases to the pre-transformed equivalents, NIBUT values were on average 1.04 times that of mTBUT, while mTBUT was 1.22 times that of TBUT, and NIBUT was 1.26 times that of TBUT.

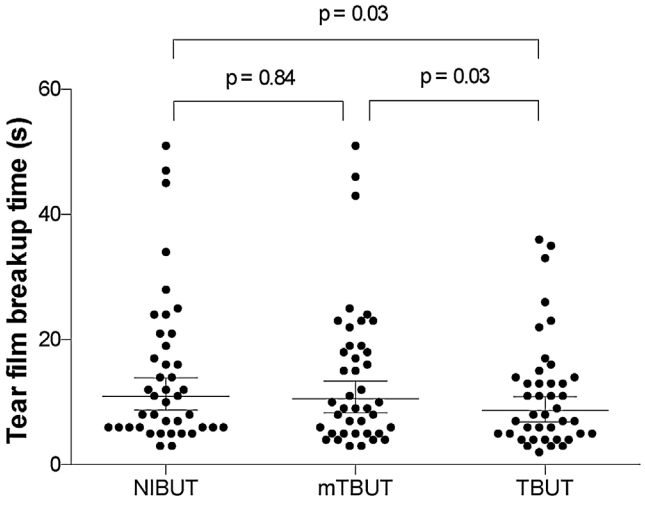

Fig. 1. The distribution of tear film break-up time measurements obtained from the non-invasive (NIBUT), minimal (mTBUT) and conventional (TBUT) fluorescein instillation techniques. Each point represents the tear film break-up time measurement of an individual eye. Bars represent the geometric mean and 95% confidence interval.

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Table 1
The distribution of tear film break-up time measurements obtained from the non-invasive (NIBUT), minimal (mTBUT) and conventional (TBUT) fluorescein instillation techniques.

	NIBUT	mTBUT	TBUT
Geometric mean, 95% CI (s)	10.9 (8.6–13.8)	10.6 (8.3–13.4)	8.6 (6.8–10.9)
Median, IQR (s)	11 (6–20)	10 (5–19)	8 (5–14)
D'Agostino-Pearson normality test (p-value)	<0.001*	<0.001*	<0.001*

Table 2
Pairwise analysis of the tear film break-up time measurements obtained from the non-invasive (NIBUT), minimal (mTBUT) and conventional (TBUT) fluorescein instillation techniques, following logarithmic transformation.

	NIBUT vs. mTBUT	mTBUT vs. TBUT	NIBUT vs. TBUT
Tukey test for means (multiplicity adjusted p-value)	0.84	0.03*	0.03*
F-test for variances (p-value)	0.87	0.04*	0.04*
Intra-class correlation coefficient, 95% CI	0.83 (0.70–0.91)	0.76 (0.56–0.87)	0.66 (0.42–0.81)
Correlation (p-value)	<0.001*	<0.001*	<0.001*
Bland-Altman bias, 95% limits of agreement	0.017 (−0.354 to 0.388)	0.085 (−0.326 to 0.496)	0.102 (−0.385 to 0.589)

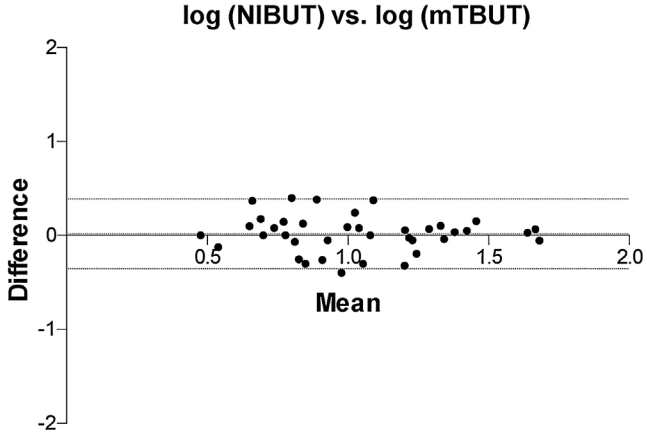

Fig. 2. Bland-Altman plot of tear film break-up time measurements obtained from the non-invasive (NIBUT) and minimal (mTBUT) fluorescein instillation techniques, following logarithmic transformation. Dotted lines represent the bias and 95% limits of agreement.

4. Discussion

Dry eye disease is a common chronic condition of the tear film and ocular surface associated with symptoms of discomfort, visual disturbance, and reduced quality of life [18,19]. Tear film instability is a central hallmark of dry eye disease, common to the variety of dry eye subtypes and aetiologies. Instability can impede the ability of the tear film to fulfil its defensive, mechanical and optical functions, and ultimately contributes towards ocular surface inflammation [4]. Consequently, tear film stability assessment through the measurement of break-up time, forms an integral part of the objective identification and evaluation of patients with dry eye [1,2]. Consistent with previous studies [14–16], tear film stability data were noted to be positively skewed, requiring logarithmic transformation prior to parametric statistical analysis. A broad range of break-up time values were obtained from all three techniques, encompassing both normal and abnormal tear film states in the subjects assessed [4].

Clinically, the measurement of tear film break-up time is frequently incorporated with routine corneal staining examinations [5–7]. It is performed following the instillation of aqueous fluorescein sodium, which improves tear film visibility under blue light, simplifying the measurement. This is commonly preferred by

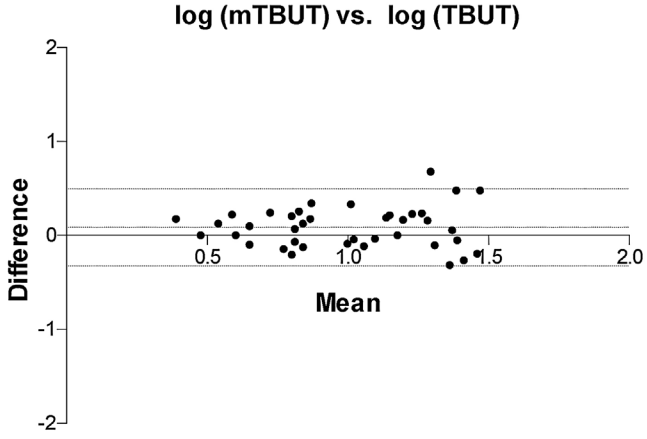

Fig. 3. Bland-Altman plot of tear film break-up time measurements obtained from the minimal (mTBUT) and conventional (TBUT) fluorescein instillation techniques, following logarithmic transformation. Dotted lines represent the bias and 95% limits of agreement.

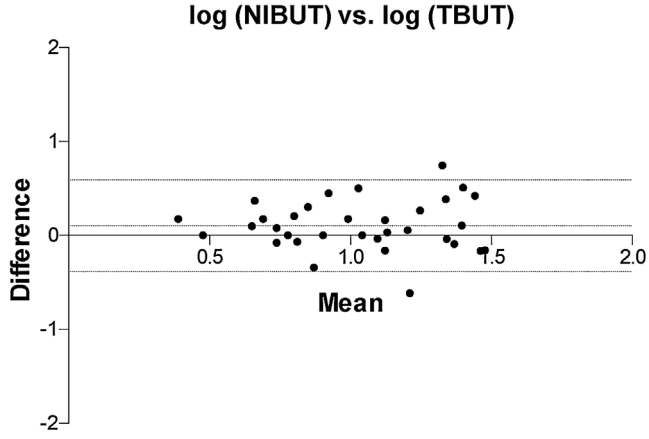

Fig. 4. Bland-Altman plot of tear film break-up time measurements obtained from the non-invasive (NIBUT) and conventional (TBUT) fluorescein instillation techniques, following logarithmic transformation. Dotted lines represent the bias and 95% limits of agreement.

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clinicians over non-invasive techniques with reflected mires [20], possibly due to the time-consuming nature, perceived complexity and additional costs in time and instrumentation associated with non-invasive measurements. However, it has been established that the application of fluorescein with conventional strips destabilises the tear film, reducing stability measures. Previous reports have shown that tear film stability values performed with non-invasive techniques tend to be longer for the same tear film [9,10]. This was consistent with the results of this study which showed that NIBUT values were significantly longer than that of TBUT (geometric mean 10.9 s vs. 8.6s, $p = 0.03$). This disparity has been attributed to both the volume of fluid introduced by the fluorescein strip, as well as the ocular surface sensation elicited, which likely triggers reflex tearing, thus introducing further error associated with TBUT measurements [11].

The Dry Eye Test™ strip has a narrowed tip relative to conventional fluorescein strips, which prevents retention of excess fluid. This allows the Dry Eye Test strips to more consistently deliver minimal fluorescein, within approximately 1 μl of fluid [11]. The results of this study demonstrated that mTBUT values were significantly longer than TBUT values (geometric mean 10.6 s vs. 8.6 s, $p = 0.03$). This contrasts with the findings of an earlier study that reported no significant differences between mean mTBUT and TBUT [11], although the naturally positively skewed distribution of the measurements affects the legitimacy of parametric analysis. The differences in instrumentation used to conduct measurements may also have contributed. Previous reports that have compared the tear film break-up times obtained with conventional strips and micropipette instillation of 1 μl of fluorescein have also yielded mixed results [15,21]. The differences detected in the current study might potentially be related to the wider range and higher mean values of the tear break-up time measurements recorded relative to those in earlier reports.

The results also show that mTBUT measurements did not differ significantly from NIBUT (geometric mean 10.6 s vs. 10.9s, $p = 0.84$). The apparent lack of tear film destabilisation from mTBUT might be attributable to the minimal invasiveness of the test, with only minute volumes of fluid instilled. The previously reported reduction in sensation upon application, and subsequent decreased potential for reflex tearing might also contribute [11]. Despite the instillation of 1 μl of aqueous fluorescein, the Dry Eye Test elicits comparable stability measures to the non-invasive test. This suggests that the reduced values obtained with the conventional fluorescein strip break-up test are a function of the volume of the fluid instilled, rather than the properties of fluorescein itself, although concentration effects cannot be entirely discounted. Interestingly, the results from the current study contrast with those of a previous report, which demonstrated that non-invasive tear film stability measurements were significantly longer than those obtained using a folded fluorescein strip, modified to decrease the amount of fluid instilled [22]. However the actual volumes instilled using the folded fluorescein strip technique were not specified, and the discrepancy in results could potentially reflect larger volumes of fluid instilled by the folded fluorescein strip, than the 1 μl delivered by the Dry Eye Test.

The F-test of variances demonstrated that the distribution of values is narrower for TBUT measurements than both NIBUT and mTBUT (all $p < 0.05$), although there were no significant differences between NIBUT and mTBUT ($p = 0.87$). This likely reflects the global reduction in tear film stability values with TBUT relative to both NIBUT and mTBUT, due to tear film destabilisation [9,10]. Clinically, this suggests that TBUT displays lesser sensitivity in discriminating between tear film stability states than either NIBUT or mTBUT.

The stability measurement techniques were found to be significantly correlated in all three pairwise comparisons (all $p < 0.001$). However, the intraclass correlation co-efficient was superior for NIBUT versus mTBUT than for the other two pairwise comparisons. Furthermore, the Bland-Altman bias was smallest for NIBUT versus mTBUT, which also displayed the narrowest limits of agreements. This indicates that NIBUT and mTBUT have higher conformity and agreement with each other, than with TBUT. The susceptibility to tear film destabilisation associated with TBUT is likely to contribute. Application of the strips by a single investigator ensured minimal variability in technique in the current study, but depending on technique, conventional fluorescein strips used for the measurement of TBUT can deliver variable amounts of fluid, between 15 and 30 μl, further reducing the precision of this technique [9,10]. The narrower tip of the Dry Eye Test strips, which causes excess fluid to fall naturally prior to application, minimises the variance in the instilled volume of fluorescein [11,23], and likely contributes to the greater reported precision of the Dry Eye Test [11], as well as its greater conformity to non-invasive techniques.

Clinicians may prefer the familiarity and ease of use of minimal fluorescein tests over non-invasive techniques which are perceived to be more challenging to interpret and require additional instrumentation. The instillation of minimal fluorescein volumes appears to offer the advantage of superior visualisation of the tear film for stability measurement, without significantly destabilising the tear film through its application. The Dry Eye Test allows for the instillation of a controlled volume of 1 μl fluorescein in the clinical setting, without the need for instruments such as micropipettes [11]. Shaking the excess fluid from wetted conventional fluorescein strips prior to application, can also faciltate some reduction in the amount of fluorescein instilled. The use of a yellow Wratten short-wavelength barrier filter can enhance fluorescein visualisation, by reducing overlap between its excitation and emission spectra [8]. This allows for superior fluorescein visualisation, even with minimal instilled volumes.

This study is not without limitations. The potential for the lack of investigator-masking and randomisation of testing order to introduce bias is acknowledged. Nevertheless, the study was adequately powered, and the instrumentation used to conduct measurements was kept consistent between all three techniques to allow for a scientifically-controlled investigation of the effect of minimising fluorescein volume instillation on tear film stability measurements.

In summary, tear film stability measurements with the conventional fluorescein strip test were shortened, while minimal fluorescein volume instillation and non-invasive techniques produced comparable readings. This suggests that minimising instilled volumes can reduce the tear film destabilising effects of fluorescein.

Conflict of interest

The authors have no conflicts of interest to declare.

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