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Comparative analysis of tear film stability: Automated non-invasive and fluorescein tear break-up time

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Summary. — The tear film plays a crucial role in maintaining ocular surface physiology, with tear film stability being a key parameter. Tear break-up time (TBUT) is a common method for assessing tear film stability, involving the duration between a full blink and the initial disruption in the tear film. This study aimed to assess non-invasive break-up time (NIBUT) using an automatic approach and compare it with the traditional fluorescein break-up time (fBUT) method. Forty-three non-contact lens wearers were enrolled, and NIBUT data were collected using the Sirius+ instrument. fBUT was conducted using fluorescein sodium strips. Test-retest measurements were performed with 2 hours delay. NIBUT values were consistently longer than fBUT values (14.8 ± 8.0 s *vs.* 8.7 ± 5.2 s), aligning with existing literature. The results revealed good test-retest reliability for both methods; however, intra-observer repeatability was weak. A potential limitation was the non-randomized sequence of measurements, with fBUT consistently performed at the end due to its invasive nature. Variations in the coverage area of fBUT and NIBUT assessments, influenced by factors like the Placido disc dimension, and the lash shadows, may have contributed to shorter times with fBUT. Preliminary analyses focusing only on the corneal area covered by Sirius+ Placido rings supported this hypothesis. In summary, while NIBUT consistently showed longer values than fBUT, further investigations considering limitations and potential influencing factors are warranted for a comprehensive understanding of tear film stability assessment.

The tear film is a thin structure deeply involved in maintaining ocular surface physiology. The assessment of tear film stability is of paramount importance [1, 2]. According to the three-layered model, the stability is maintained by the prevention of evaporation (lipid layer), the increase of volume and lubricity (aqueous layer), and the reduction of corneal epithelium hydrophobicity (mucin layer). The absence of stability can be assessed using tear break-up time (TBUT), which represents the duration between the conclusion of a full blink and the onset of the initial disruption in the tear film [1, 2]. In 1969, it was introduced the first procedure to assess the TBUT, named fluorescein

BUT (fBUT), which allowed identifying breaks in tear film coloured with sodium fluorescein dye through the use of a biomicroscope and cobalt blue light [3]. Despite the widespread use of fBUT as a common test for assessing tear film, its clinical reliability has been widely acknowledged as poor [4,5], primarily due to the invasiveness associated with fluorescein [6]. Various modifications to the fBUT procedure have been proposed to enhance reliability. However, the ideal method for evaluating tear film stability should involve a non-invasive approach to prevent alterations to the tear film [2,7]. NIBUT, or non-invasive break-up time, represents the time between the completion of a full blink and the appearance of a disruption or break in the reflected image of a mire or grid pattern on the anterior surface of the tear film [1,6]. NIBUT has gained widespread adoption and has been incorporated into modern corneal topography systems. In these systems, automated algorithms assess NIBUT from video data obtained through videokeratography. Variations in Placido disc characteristics, background illumination, and algorithms can lead to differences in the obtained results. The present study was aimed to evaluate the agreement and repeatability of a recently developed automated topography-based NIBUT in comparison with fBUT.

The study involved forty-three participants (sixteen males and twenty-seven females), exclusively non-contact lens wearers, with an average age of 23.1 ± 2.1 years. Inclusion criteria were the absence of ocular pathology, refractive surgery, ocular drug treatment, known general pathology and medication impacting the ocular surface. Participants were also required to demonstrate the ability and willingness to adhere to study protocols. NIBUT data were collected using Sirius+ (CSO, Florence, Italy), functioning as a Placido disc topographer integrated with a Scheimpflug tomographer. Sirius+ employed an automated algorithm to analyze disruptions in the projected ring structure over time. Only changes persisting until the end of the recording were considered break-ups, and the algorithm provided the first break-up topographically for each sector. The fluorescein break-up time (fBUT) procedure was conducted using fluorescein sodium strips (I-DEW FLO, Endot, UK) following the procedure outlined by Pult Riede-Pult [8], using a slit lamp (HR Elite, CSO, Florence, Italy) equipped with blue cobalt and yellow filters. The fBUT procedure was recorded using the digital slit lamp. All measurements were performed in the same lab by the same researcher. Three repeated measurements were carried out for each procedure in the right eye. fBUT was carried out always at the end due to its invasiveness compared to the NIBUT measurements. Participants, as for non-invasive devices, were instructed to blink twice and then refrain from blinking for as long as possible. The reliability of the test-retest was assessed by conducting the identical series of measurements, for each specific subject, on the same day, with a minimum interval of 2 hours after the initial set of measurements. A different researcher received instructions to play the fBUT videos to identify the initial break-up, allowing for the option to rewind the video for better identification of the break-up. Regarding the Sirius+, only the first NIBUT, defined as the first disruption of the projected rings regardless of the sector, was directly obtained through automatic algorithm analysis. All data sets did not result normally distributed (Shapiro-Wilk test; $p < 0.005$), thus non-parametric statistics were used. The agreement among the BUT assessment procedures was investigated by a matched comparison (Wilcoxon signed-rank test). Spearman correlation was also calculated. Intra-observer repeatability was evaluated with the coefficients of precision (CP), repeatability (CR), and variation (CV). Test-retest reliability, involving the mean of the three measures at the test and the mean of the three measures at the retest, was evaluated through the Intraclass Correlation Coefficient (ICC) based on mean measurement, absolute agreement, and a two-way mixed effects model. Additionally, a

Table I. – *NIBUT measures as a function of the order of measurement (test and retest averaged). Overall value is also reported.*

	Average of first NIBUT measure at test and retest (s)	Average of second NIBUT measure at test and retest (s)	Average of third NIBUT measure at test and retest (s)	Overall NIBUT measure (s)
Mean of participants	13.6	15.0	15.8	14.8
SD of participants	8.3	8.7	9.7	8.0

comparison between the test and retest was executed using the matched-pairs Wilcoxon test. All statistical analyses were performed with SPSS version 2.8 (IBM SPSS Statistics, USA).

The trend of the measures achieved from the first to the last are reported in tables I and II for NIBUT and fBUT, respectively. The overall measure for the two procedures (average of test and retest $\pm$ SD) resulted in 14.8 ± 8.0, and 8.7 ± 5.2 s with the Sirius+, and fluorescein-based procedure, respectively. Paired comparison showed significant difference ($p < 0.001$). Correlation between the two procedures resulted in significance ($p < 0.001$). Intra-observer repeatability for the two instruments, in the two sessions, was notably weak, evident from the elevated values of CP, CR, and CV as presented in table III. The test-retest results are presented in table IV. Notably, the ICC values were substantial (ranging from 0.61 to 0.78) for both the Sirius+ and the fBUT. No significant test-retest differences were observed.

The study showed that NIBUT values were longer than fBUT, aligning with findings in existing literature [6, 9-11]. Notably, other studies have reported shorter automatic NIBUT compared to fBUT in the literature as well [12, 13]. In the current study, it is important to note a potential limitation regarding the difference between fBUT and NIBUT. The sequence of the measurements was not fully randomized, with fBUT consistently performed at the end due to its invasive nature. Additionally, another factor

Table II. – *fBUT measures as a function of the order of measurement (test and retest averaged). Overall value is also reported.*

	Average of first fBUT measure at test and retest (s)	Average of second fBUT measure at test and retest (s)	Average of third fBUT measure at test and retest (s)	Overall fBUT measure (s)
Mean of participants	8.3	9.2	8.8	8.7
SD of participants	4.6	5.8	6.0	5.2

TABLE III. – *Coefficient of precision (CP), coefficient of repeatability (CR) and coefficient of variation (CV) for the measures in the first session (test) and in second session (retest).*

	Test	Retest
Sirius+	CP=13.8 s CR= 19.6 s CV=0.45	CP=14.3 s CR= 20.3 s CV=0.53
fBUT	CP=7.1 s CR= 10.0 s CV=0.41	CP=6.1 s CR= 8.6 s CV=0.36

contributing to shorter times with fBUT may stem from the varied coverage area assessed by fBUT and NIBUT procedures. In the NIBUT procedure, the Placido rings were reflected in a limited corneal area. Moreover, although participant was requested to open widely his/her lids, part of this area could be covered by lash shadows, limiting the processing of the automated algorithm. Therefore, the NIBUT area of assessment was reduced compared to the fBUT procedure. Consequently, the fBUT procedure was able to identify breaks in zones not covered by the NIBUT procedure possibly resulting in shorter BUT. Regarding this aspect, preliminary analyses were conducted on a randomly selected subset of thirty-four measurements. fBUT videos were re-examined and new measurements were carried out considering only the corneal area covered (in the same subject) by the reflected Placido rings of the Sirius+. In this subsample, times resulted in $14.3 \pm 8.3\,\text{s}$, $8.7 \pm 6.2\,\text{s}$, and $11.7 \pm 8.2\,\text{s}$ for NIBUT with Sirius+, fBUT performed in the whole area, and fBUT with limited area respectively. Differences between Sirius+ and fBUT performed in the whole area resulted still significant (Wilcoxon test; $P < 0.001$). However, differences between Sirius+ and fBUT performed in a limited area were not significant (Wilcoxon test; $P = 0.08$).

TABLE IV. – *Test-retest analysis for 43 participants: descriptive statistics for BUT in seconds, Intraclass Correlation Coefficient (ICC) between test and retest measures, and p-values (Wilcoxon test) from paired comparisons between test and retest.*

	Test (s) Mean ± SD	Retest (s) Mean ± SD	ICC [95% confidence intervals] (p-value)	Comparison (p-value of Wilcoxon-test)
Sirius+	15.8 ± 9.5	13.8 ± 8.1	0.78 [0.59 – 0.88] ≤ 0.001	0.08
fBUT	8.9 ± 6.0	8.6 ± 6.2	0.61 [0.28 – 0.79] ≤ 0.01	0.38

In conclusion, the study focused on the assessment of NIBUT automatically, comparing it with the fBUT method. The results indicate that NIBUT values were consistently longer than fBUT, aligning with existing literature. The study demonstrated substantial test-retest reliability for both Sirius+ and fBUT. However, intra-observer repeatability resulted in weakness for both procedures. A potential limitation in the study was the non-randomized sequence of measurements, with fBUT consistently performed at the end due to its invasive nature. Additionally, the different coverage areas of fBUT and NIBUT assessments, influenced by factors like Placido disc dimension and lash shadows, potentially contributed to shorter times with fBUT. Preliminary findings to test this hypothesis support it. In summary, while NIBUT demonstrated longer values than fBUT, further investigations considering the limitations and potential influencing factors are warranted for a comprehensive understanding of tear film stability assessment.

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