REPEATABILITY OF NONINVASIVE BREAK-UP TIME MEASUREMENTS USING KERATOGRAPH OCULUS 3

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The authors of the paper declare that the procedures used in the study correspond to the ethical principles of the Declaration of Helsinki from 1975 (revised in 2000).

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SUMMARY

Aim: The primary aim of this study is to evaluate the repeatability of noninvasive break-up time (NIBUT) measurement by keratograph when it is determined from one, two or three partial measurements, and to recommend a suitable methodology for practice. Another goal is to verify that repeated measurements do not affect the measured value.
Material and Methods: Thirty-eight healthy volunteers (30 women and 8 men) aged between 19 and 50 years old were included in the study, in which only one eye of each volunteer was measured. The study was designed as a prospective one. Each subject adapted to the local conditions of the laboratory for 15 minutes and subsequently underwent two series of NIBUT measurements (test, retest) on an OCULUS 3 Keratograph. The minimum time interval between the two series was 10 minutes, in which each series contained three partial measurements approximately 3 minutes apart. The resulting NIBUT in each series was determined (A) as the first value in the given series, (B) as the average of the first two, or (C) all three measurements in the given series. Repeatability was assessed by a Bland-Altman analysis and expressed as a repeatability coefficient. In every case, only the time of the first break-up of the tear film was monitored.
Results: The statistical analysis did not show statistically significant differences both between partial measurements of NIBUT in the individual series ($p = 0.92$, $p = 0.81$) and when comparing all six measurements ($p = 0.95$). The mean values of the partial measurements ranged from 13.6 s to 14.4 s. The repeatability coefficients were found to be 15.0 s, 12.1 s and 10.0 s for methodologies A, B and C, respectively. A supplementary analysis for 12 eyes with low NIBUT (< 10 s) showed statistically significantly better repeatability in this group, with coefficients of 7.0 s (methodology A), 6.0 s (B) and 4.6 s (C).
Conclusion: Determination of NIBUT from three consecutive measurements (with a sufficient interval of ideally a few minutes) significantly improves repeatability. Such repeated NIBUT measurements do not have a significant effect on the measured value. The mentioned methodology for measuring NIBUT on a keratograph can be recommended for use in practice.
Key words: break-up time test, BUT, NIBUT, repeatability of measurement, tear film, dry eye syndrome

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INTRODUCTION

The tear film represents the first protective barrier of the anterior segment of the eye from the external environment, and at the same time plays a fundamental role in the nutrition of the cornea and lubrication of the ocular surface. Another of its functions is to ensure a perfectly smooth surface, by which it contributes significantly to quality and sharp vision. Reduced quality and quantity of the tear film may lead for example to dry eye disease (DED), which can substantially reduce quality of life [1]. At the same time, the prevalence of disorders and complaints in relation to the tear film is increasing at present, for example in connection with ever increasing and longer dwelling in air-conditioned environments, wearing contact lenses or undergoing corneal laser refractive surgery [2,3].

An important element in the diagnostic examination of the tear film is measurement of its break-up time (BUT) [4]. The standard method for determining BUT is subjective evaluation of the moment of break-up, when the tear film is made visible through the application of fluorescein staining (invasive BUT, IBUT). However, such a procedure may initiate reflexive lachrymation and places a burden on the patient under examination [5–8]. To a considerable extent, the above-stated undesirable phenomena can be avoided through the use of a noninvasive method of BUT examination (NIBUT) [9,10]. Stipulation of NIBUT takes place on the basis of an evaluation of a video recording of an image of the Placido rings projected onto the anterior surface of the cornea. Break-up of the tear film is manifested in a disruption of this image, which is automatically detected in the video by the relevant software. An example of a device which uses this method is the keratograph OCULUS version 3 and higher.

A disadvantage of measurement of BUT and NIBUT is that a single measurement may be subject to a considerable margin of error. As a result, in the case of BUT it is therefore recommended to determine the resulting value as the average of three consecutive partial measurements [11]. The aim of our study was to verify whether this method or a simpler modification thereof is appropriate also for examination of NIBUT on the keratograph OCULUS 3. At the same time the study aimed to observe whether or not the necessary repetition of partial NIBUT measurements had an influence on the condition of the tear film.

MATERIAL AND METHOD

Cohort of probands

The study included 38 volunteers (30 women and 8 men) aged between 19 and 50 years (average age 24 ±8 years), in whom only one eye was tested in each subject. Any persons suffering from abnormalities of the anterior segment of the eye were excluded from the study. The exclusion criteria also included the use of any preparations on the day of measurement which could have an influence on the tear film. Contact lens wearers were asked to remove their contact lenses for at least 24 hours before the measurement. The study was governed according to the principles of the Helsinki declaration, in which each participant was familiarized in detail with the course of the study before taking part, and signed an informed consent form for participation in the study.

Measurement procedure

The study was conducted as a prospective study, the repeatability of measurements was evaluated by the test and retest method. Before the actual measurement, each of the participants spent 15 minutes in the examination room in order to adapt to the local conditions. During this time, they were familiarized with the course of the examination. Subsequently two series of NIBUT measurements were conducted (first series – test, second series – retest), in which each incorporated three partial measurements. NIBUT was measured in the keratograph OCULUS 3 (Oculus, Wetzlar, Germany). A time interval of at least 10 minutes was adhered to between both series (on average 14 ±4 min.), and approximately 3 minutes between the partial measurements in the individual series. The time of the first break-up of the tear film was recorded in each partial measurement. Throughout the entire time of their participation in the study, the probands were in a resting, seated position, and were prohibited from performing any close-up activity (especially working with a mobile telephone, laptop computer, reading). All measurements were performed by a trained examiner. The humidity in the examination room throughout the course of the entire experiment reached values of 31 ±6 %, the temperature 22 ±1 °C, with no significant changes during the measurement of a single proband.

The actual measurement of break-up of the tear film on the OCULUS 3 topograph was automatically commenced after a double blink, which the examined person was called upon to do, and was conducted for a maximum time of 24 s (i.e. a time of 24 s was always attributed to higher NIBUT values). If further blinking occurred between the detected break-up of the tear film, this measurement was replaced with another. During the examination, the participant followed the fixation light of the instrument with the chin and forehead placed on a headrest.

Data analysis

The resulting NIBUT value was determined by three methods:

- A – as the first measured partial value in the given series,
- B – as the average of the first two partial values in the given series,
- C – as the average of all three partial values in the given series.

The normality of distribution of the evaluated data was tested using a Shapiro-Wilk test. It was determined that the statistical distribution of data from all the partial measurements and average values from the first two or all three partial measurements in both series deviate from normal distribution. As a result, for pair comparison a Wilcoxon paired test was used, and for simultaneous comparison of more than two values a Friedman non-parametric test was used.

The repeatability of measurements of the time of the first and average break-up of the tear film for all three methods of its determination (A, B, C) was evaluated using the method of a Bland-Altman analysis, which graphically illustrates the dependency of the differences in the values of the test (values determined in the first series of measurements) and the retest (values from the second series of measurements) on their average [12]. The limits of repeatability were delineated by the boundaries of a 95% confidence interval of the differential data (test – retest), in which the upper and lower limit were determined as an average difference of ±1.96·SD, in which SD is the standard deviation of the differential data. The value of 1.96·SD represents the "coefficient of repeatability" (CoR). For the individual limits of repeatability, the precision of their estimate was also determined in the form of a 95% confidence interval [13], graphically represented by error bars. The differences in repeatability between the compared methods can be considered statistically significant, at least provided that these intervals do not overlap for one limit.

The evaluated data were represented by the average value, the standard deviation and in the case of disruption of normality also by the median and the 1st and 3rd quartile. In the text the averages and standard deviations are presented in the form of average ± standard deviation. The level of significance for all the used statistical tests was 0.05. The statistical calculations were performed in the program STATISTICA 13.4 (TIBCO Software Inc., Palo Alto, CA, USA), or in the program MS Excel 2016 (Microsoft Corporation, Remond, WA, USA).

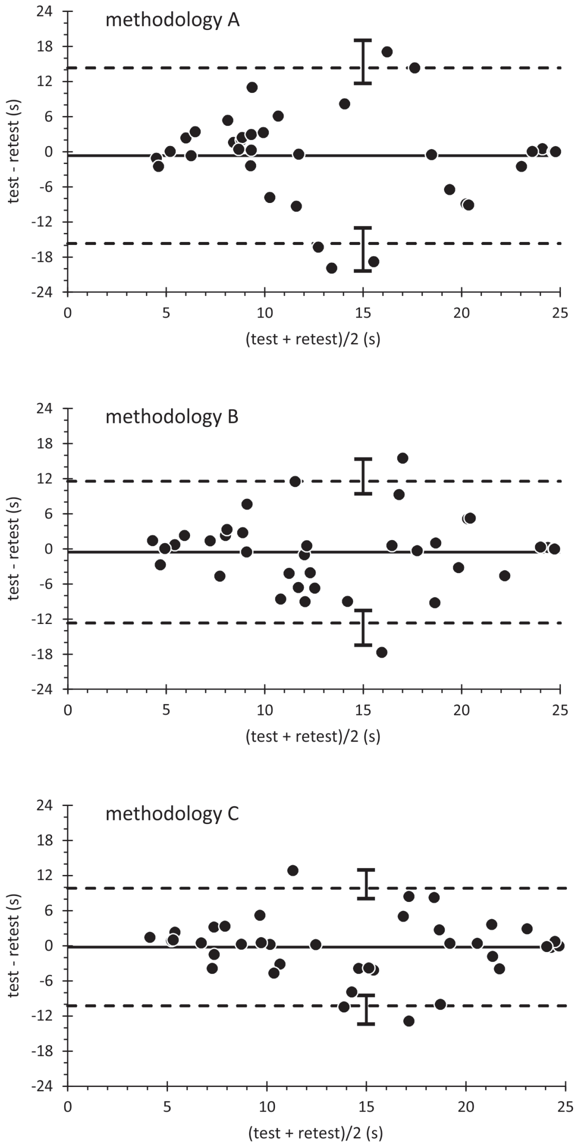

Figure 1. Bland-Altman plots depicting the dependence of NIBUT differences from test and retest on the average value in the case of individual methodologies of its determination (**A** – one measurement, top graph; **B** – average of two consecutive measurements, middle graph; **C** – average of three consecutive measurement; bottom graph). The circles represent difference values of individual eyes, the solid line represents the mean difference, the dashed lines define the 95% confidence interval of difference values and the error bars indicate the accuracy of determination of this interval

RESULTS

The statistical analysis did not demonstrate any significant differences between the partial measurements of NIBUT, either upon comparison of all six measurements ($p = 0.95$), or upon separate evaluation of the three measurements in the individual series ($p = 0.92$, $p = 0.81$). Similarly, no difference was demonstrated between the NIBUT values from the test and retest for all three considered methods of its determination ($p = 0.97$ for method A, $p = 0.77$ for B and $p = 0.88$ for C). The statistical characteristics of the corresponding data are summarized in Table 1.

The Bland-Altman graphs of the repeatability of measurements of NIBUT determined by methods A, B and C are illustrated in Figure 1. In accordance with the above-presented insignificant results of the statistical comparison of data, the average differences between the test and retest were within a range very close to zero (A: -0.7 ±7.7 s; B: -0.6 ±6.2 s; C: -0.2 ±5.7 s). It is evident from the graphs in Fig. 1 that the dispersion of values between the test and retest in all the considered methods of determining NIBUT is appreciably smaller for low values (typically < 10 s). With regard to the clinical significance of such low values, a Bland-Altman analysis was therefore additionally performed separately for eyes in which the average of all six measurements was < 10 s (total 12 eyes). The coefficients of repeatability CoR of the individual methods in the case of the entire cohort and for low NIBUT are summarized in Table 2. Significant elements of the Bland--Altman graphs (average value, limit of repeatability and the precision of their determination) are synoptically graphically compared in Figure 2, again for the entire cohort and for low NIBUT. It is evident from the graphs and the CoR values that in the case of analysis of the entire cohort, NIBUT determined on the basis of a single measurement (i.e., method A) has the worst repeatability (i.e., the widest limits of repeatability and the highest CoR), whereas by contrast the best results were obtained in an average of three consecutive measurements (method C). The difference in CoR between the two methods

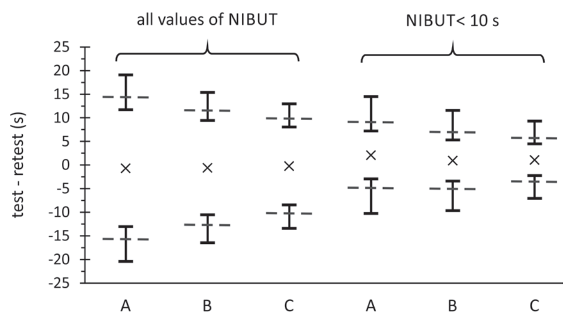

Figure 2. Graphical comparison of the essential parameters of the Bland-Altman analysis of the repeatability of the NIBUT examination for all eyes (left) and for eyes with low NIBUT (< 10 s, right) in the case of individual methodologies of its determination (**A** – one measurement; **B** – average of two consecutive measurements; **C** – average of three consecutive measurement). The crosses represent the average differences, the dashed lines define the 95% confidence interval of difference values and the error bars indicate the accuracy of determination of this interval

Table 1. Statistical characteristics of test and retest in the case of individual methodologies for NIBUT determination (**A** – one measurement; **B** – average of two consecutive measurements; **C** – average of three consecutive measurement); SD represents the standard deviation

Set	Test			Retest		
Methodology	**A**	**B**	**C**	**A**	**B**	**C**
Mean (s)	13.6	13.7	14.2	14.3	14.2	14.4
SD (s)	7.5	7.1	6.7	8.1	7.1	7.0
Median (s)	11.2	10.9	12.6	10.5	14.7	14.3
1st quartile (s)	7.4	8.0	8.9	7.7	7.0	8.2
3rd quartile (s)	20.9	19.7	20.3	23.8	18.6	20.2

Table 2. Values of coefficients of repeatability (CoR) for all eyes and for eyes with low NIBUT (< 10 s) in the case of individual methodologies of NIBUT determination (**A** – one measurement; **B** – average of two consecutive measurements; **C** – average of three consecutive measurement)

Methodology	A	B	C
CoR for all values of NIBUT (s)	15.0	12.1	10.0
CoR for low NIBUT (s)	7.0	6.0	4.6

is 5 s. From a statistical perspective, the difference between methods A and C is indicated by a virtually zero overlap of the intervals of precision (i.e., error bars in the graph) of the lower limits of repeatability. Even in the case of low NIBUT, the CoR progressively decreases from method A to method C, though the differences are not so pronounced (CoR differs by 2.4 s between A and C). A comparison of CoR determined for all data and for low NIBUT (see Table 2) together with a graphic comparison of the limits of repeatability (Fig. 2) confirms that the repeatability of measurements is significantly better for low values of NIBUT as against an analysis of the entire cohort.

From the graphs in Fig. 1 it is also possible to observe better repeatability in NIBUT values of around 20 s and higher. This is probably due to the limited measurement time, in which all NIBUT values above 24 s were uniformly attributed with a value of 24 s.

DISCUSSION

The BUT value enables us to assess the quality of the tear film and is an important parameter in the diagnosis of DED. For it to be possible to use the obtained results safely in clinical practice, especially in the diagnosis and subsequent evaluation of the impacts of any applicable intervention, it is necessary for the actual measurement to be repeatably within the framework of clinically acceptable limits. In general, repeatability can be improved by means of an averaging of a number (e.g. 3) of measurements, as is usual in the case of IBUT measurements with the use of fluorescein [11]. However, certain studies have highlighted the fact that repeated measurement in this method can influence the result [14,15]. This can be explained by the invasiveness of the procedure, in which the first application of fluorescein may irritate the eye more than the following applications [14]. By contrast, in NIBUT no colorings or pharmaceuticals are used, and the risk of irritation of the eye is therefore substantially lower. In accordance with this presupposition, in repeated measurement our study did not manifest any significant differences in NIBUT, even after the performance of 6 repetitions. It is therefore safe to use the method of averaging a number of consecutive measurements in the case of NIBUT. At the same time, our results demonstrate that use of the average of a number of measurements genuinely leads to an improvement of repeatability – this improvement was most evident upon an averaging of three measurements. Similar conclusions can be drawn also from further studies, although they did not engage explicitly with the specific impact of the averaging of the results on repeatability. For example, a publication [16] which works with similar NIBUT values (approximately 13 s) as our sample (see Table 1) states CoR values very close to ours (13.2 s for determination of NIBUT from a single measurement and 10.3 s for an average of three measurements).

Another fundamental finding is the manifest dependency of the distribution of data of the test and retest on the NIBUT value, which is evident from the course of the Bland-Altman graphs (see Fig. 1), in particular the decrease in size of the deviations from the NIBUT value of < 10 s. A similar course can be recorded also in other studies, e.g. [15–17]. In this it is precisely the value of 10 s that is generally considered the normal time limit of the first break-up of the tear film [18]. It is therefore desirable for the measurement to be as precise as possible within this area. A supplementary analysis of our data genuinely confirmed that the repeatability of NIBUT measurement using an OCULUS 3 keratograph is significantly better in this area in comparison with higher values. This fact is also confirmed by studies [19,20] comparing eyes with a normal tear film and with DED – eye with DED (therefore with a lower BUT) manifested better repeatability. By contrast, another article [21] states the opposite effect – worse repeatability in eyes with DED. However, in this case the compared measurements were conducted on different days, and the greater variability of NIBUT thus most probably attests to the increased variability of the parameters of the tear film over time in the case of DED.

Although NIBUT and IBUT show a high mutual correlation [22–25], due to the invasiveness of IBUT their values differ slightly – most publications state slightly higher values in NIBUT [22,24,25], an exception being study [23]. It is therefore always advisable to state which method was used to measure BUT. However, in terms of the repeatability and variability of data, it appears that both approaches achieve similar values [24]. From the results of study [15] it is possible to calculate CoR for one measurement of around 7 s, for an average of two measurements approximately 6 s, in which the great majority of eyes had NIBUT of less than 10 s. This data correlate well with our own data for low NIBUT.

A limitation of our study is the relatively small number of eyes with low NIBUT values, which may have a negative impact on the precision of determination of the CoR and the significance of the results. In addition, with regard to the determined dependency of CoR on the size of NIBUT, the comparison of the specific CoR values we found with data from other studies is limited if the average NIBUT values differ more markedly. A certain limitation is also represented by the use of an older version of the keratography (OCULUS 3), since the majority of the compared studies use the newer version OCULUS 5M. This enables the virtually complete elimination of glare during the examination, and may theoretically attain better results. The impact of the repeated measurements upon the use of the 5M version should therefore be smaller, and the proposed method of three measurements can be recommended also in this case.

CONCLUSION

Based on our results as well as the findings from other available studies, for determination of NIBUT with the aid of an OCULUS keratograph it is possible to recommend, if possible, the performance of three consecutive measurements (with a sufficient interval, ideally of several minutes), and to consider their arithmetical average as the resulting value. This determined value generally demonstrates manifestly better repeatability than one simple measurement. NIBUT represents an equivalent substitute for the classic procedure using fluorescein, though by contrast with this it does not place any significant burden on the patient and only minimally influences the stability of the tear film.

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