COMPARISON OF NITBUT ANDTBUT TECHNIQUES FOR THE ASSESSMENT OF THE TEAR FILM STABILITY

RADHIKA VEERAPANENI AND ANITHA KANNAN
OPTM 797
SENIOR PROJECT

MICHIGAN COLLEGE OF OPTOMETRY
1310, CRAMER CIRCLE
BIG RAPIDS, MICHIGAN 49307

COMPARISON OF NITBUT AND TBUT TECHNIQUES FOR THE ASSESSMENT OF THE TEAR FILM STABILITY

ABSTRACT:

The purpose of this study is to compare the non-invasive tear break-up time (NITBUT) and the invasive tear break-up time (TBUT) and to determine if the NITBUT can be used instead of the TBUT to assess the tear film stability. There have been concerns regarding the validity of the results obtained by the conventional TBUT technique. Recently several non-invasive techniques have been proposed as an alternative. The NITBUT was measured using the tearscope without the instillation of flourescein. The TBUT was measured by the conventional technique using sodium flourescein and the aid of a slit lamp biomicroscope. These techniques were done on normal subjects and subjects with dry eye. The results from this study suggest that there appears to be some significant difference ($P \leq 0.02302$) between the tear film stability observed by NITBUT and TBUT methods for all subjects (with and without dry eye). This difference appears to be more prominent for the subjects diagnosed with dry eye ($P \leq 0.03906$). For the normal subjects, there appears to be no significant difference between the means of the NITBUT and TBUT values ($P \leq 0.1454$). However, it is not clear if the significant difference between the NITBUT and the TBUT is consistent or not for all subjects.

INTRODUCTION:

The tear break-up time is defined as the interval between a complete blink and the first appearance of a black spot in the fluorescein-stained tear film. This value, in seconds, has been used as a measure of tear film stability. The instillation of flourescein onto the open eye has been the only means of clinically measuring the precorneal tear film stability (*Mengher et al., 1985*). Although this method has been widely used for the assessment of tear film stability there has been some controversy over the validity of using this invasive technique. For instance, this technique suffers from certain drawbacks in assessing the pre-corneal tear film, the major one being the invasive nature of the test.

In recent years, several noninvasive tear break-up time (NITBUT) techniques have been proposed. This non-invasive technique provides an alternative means of assessing the formation and stability of the tear film (*Mengher et al., 1985*). This method is based on observing changes in the reflected image of a grid projected onto the open eye. A well-defined grid implies the presence of a stable and intact tear film. These lines were observed to become distorted and discontinuous randomly on destabilization of the tear film, representing changes in the tear film. The distortion represents a break in the pre-corneal tear film. The time taken for the appearance of the first randomly located distortion in the grid image was therefore a measure of the tear film break-up time (*Mengher et al., 1985*).

Patel et al., (1985) used a noninvasive technique on 6 subjects to measure the tear thinning time (TTT) before and after instillation of fluorescein. They found the mean TTT to be statistically significant and concluded that the instillation of fluorescein destabilizes the tear film. *Mengher et a*l *(1985)* compared the NITBUT of 9 subjects pre- and post fluorescein instillation and found that the tear break-up time values decrease after the instillation of fluorescein. The study by *Cho et al (1996)* on 24 asymptomatic Hong Kong-Chinese did not agree with the conclusions of *Patel et al* and *Mengher et al.* They concluded that fluorescein did not cause any significant change in the NITBUT. However, it is conceivable that the tear film properties responsible for its stability in the open eye may be altered following the instillation of fluorescein.

There have been reports that indicate that the NITBUT values are generally higher than invasive tear break-up time (TBUT) values (*Cho and Douthwaite., 1995*). Brown et al *(1993)* found that although the average NITBUT value was longer than the average TBUT value, there was no significant difference between the two values. The exact relationship between the TBUT and NITBUT still remains unclear. Based on previous studies it has been suggested that measuring TBUT or NITBUT of a subject on a single visit may not give an accurate assessment of the state of the subjects tear film stability (*Cho and Douthwaite., 1995*). The aim of this study was to compare the effects of two different techniques (invasive and noninvasive) on tear break-up time in 27 subjects.

MATERIALS AND METHODS:

Subjects:

Fifteen optometry students (9 females; 6 males) from the Michigan College of Optometry, aged 21 to 32 years old participated in this study. Five of the fifteen students were diagnosed with dry eyes. The students who wore contact lenses were requested not to wear their lenses for a minimum of twenty-four hours prior to taking the measurements.

Procedure:

All measurements were made in a quiet, dimly lit examination room. TBUT and NITBUT measurements were taken on each subject on different days.

TBUT Test:

The subject was seated at a slit lamp biomicroscope with the chin on the chin rest and the forehead firmly pressed against the forehead rest. A wide beam with the cobalt blue filter was used so that the whole cornea was illuminated, and viewed with 10x magnification. Flourescein was applied to the eye using a moistened Ful-Glo flourescein strip with excess moisture shaken off. The strip was applied to the bulbar conjunctiva of the eye tested. The subject was asked to blink three times to distribute the flourescein, and then refrain from blinking for as long as possible. The subjects were instructed to blink when any feeling of discomfort was felt, in order to avoid reflex tearing. A

stopwatch was started immediately after the last blink and stopped at the first appearance of black spots or streaks. This time interval was recorded as the TBUT. Three readings were recorded for each eye. The subject was given a minimum 10-minute break between each eye.

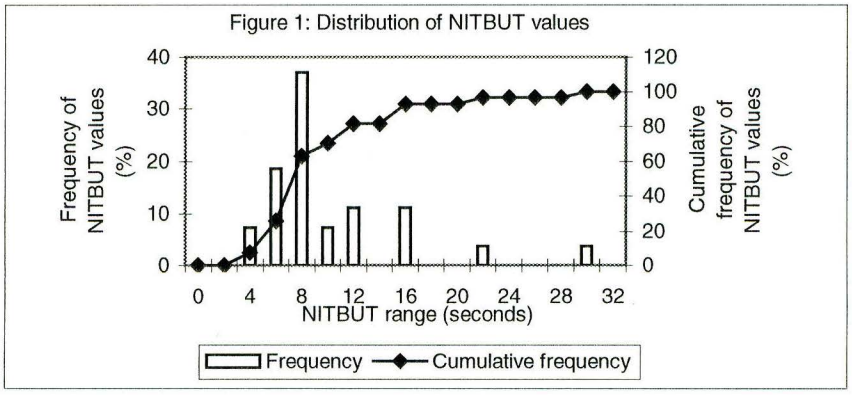
NITBUT Test:

The NITBUT was measured using the Tearscope-plus. The tearscope consists of a uniformly illuminated tube onto which the coarse grid pattern was placed with the narrow side inserted first. A 10-diopter lens was secured to the observation aperture at the back of the tearsope-plus.

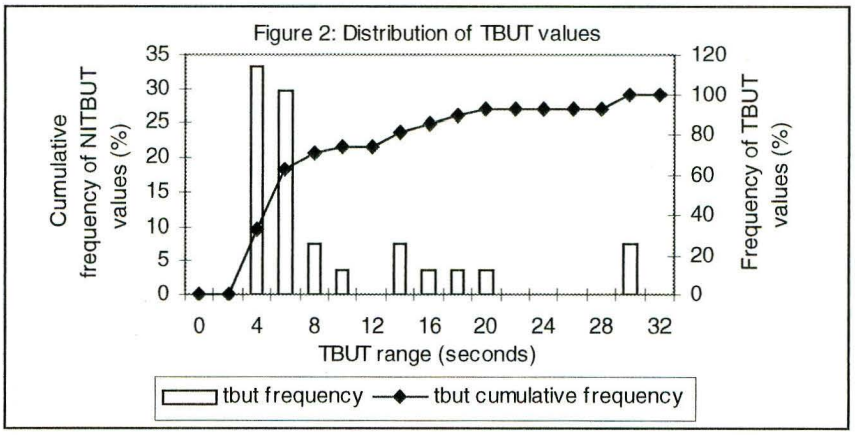
The subject was asked to blink three times and try not to blink for as long as possible. The reflected image of the coarse grid pattern was viewed. The subject was instructed to blink when any discomfort was felt, to minimize reflex tearing. The NITBUT was measured using the timer on the instrument, as the time from the last blink to the first appearance of distortion of the lines of the reflected image. Three readings were recorded for each eye with a minimum delay of 10 minutes between each eye.

RESULTS AND DISCUSSION:

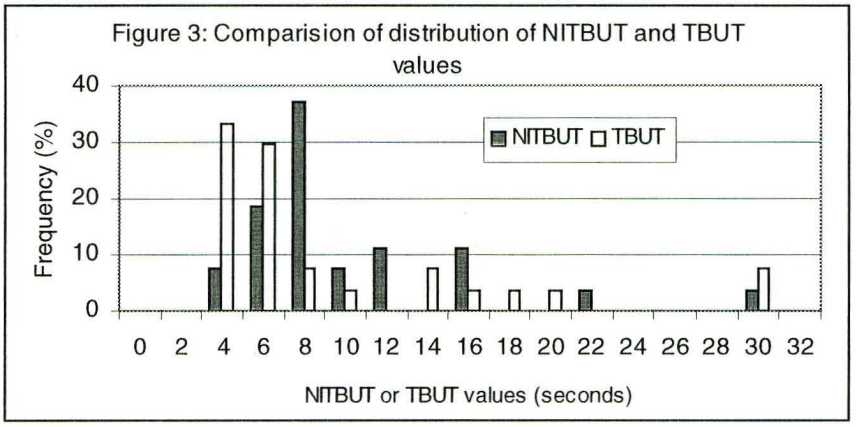
The distributions of NITBUT and TBUT values are shown in Figures 1 and 2. NITBUT values of approximately 37% of the subjects are in the range of 8 to 10 seconds and approximately 19% of the subjects have NITBUT values in the range of 6 to 8 seconds. Around 11% of subjects have NITBUT values in the range of 12 to 14 seconds while another 11% have NITBUT values in the range of 16 to 18. The cumulative distributions of the NITBUT values are also shown in Figure 1. 70% of the subjects have NITBUT values below 12 seconds.



Approximately 33% of the subjects have TBUT values in the range of 4 to 6 seconds and approximately 30% of the subjects have TBUT values in the range of 6 to 8 seconds (Figure 2). Around 7.5% of subjects have TBUT values in the range of 8 to 10 seconds while another 7.5% have TBUT values in the range of 14 to 16. The cumulative distributions of the TBUT values are also shown in Figure 2. 70% of the subjects have TBUT values below 10 seconds.

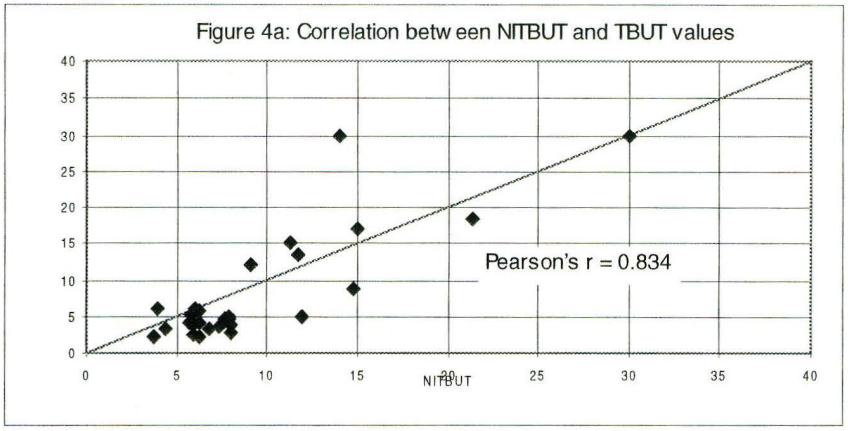


The distributions of NITBUT and TBUT values are plotted in Figure 3 for direct comparison. It appears that NITBUT values are typically higher than the TBUT values. This could be the result of fluorescein reducing the stability of the tear film (*Mengher et al, 1985*).

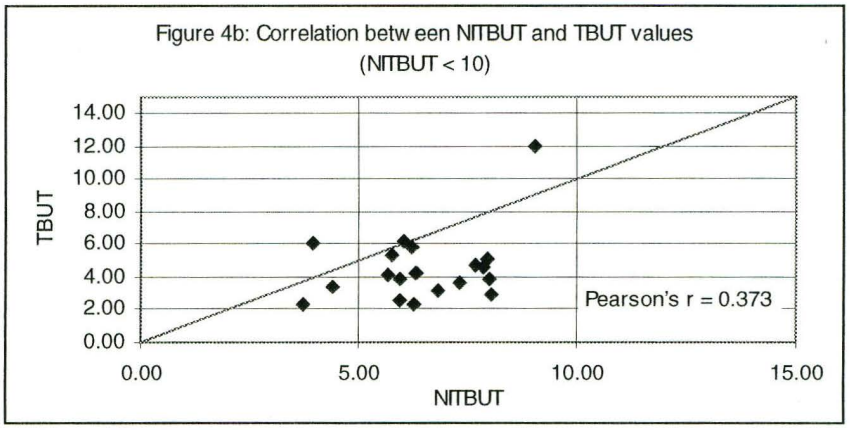


To evaluate the possibility of either a positive or negative correlation between the two values, the values of TBUT are plotted against NITBUT in Figure 4a. The line of perfect correlation representing perfect positive correlation is also shown in Figure 4a. Data points above the line of equality indicate subjects for whom TBUT values are greater than NITBUT values, while the points below the line indicate subjects whose

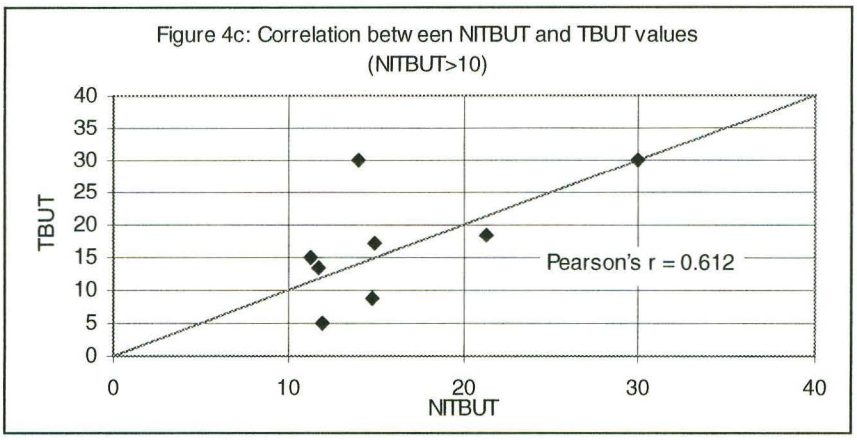
NITBUT values are greater than TBUT values. It appears that NITBUT values are greater than the TBUT values for subjects with NITBUT values less than 10. For subjects with a NITBUT value greater than 10, there appears to be no such pattern. To verify the correlation between NITBUT and TBUT values, Pearson's product moment correlation coefficient, r, has been calculated for all the measurements, as well as for the cases where NITBUT is less than 10 seconds and for NITBUT in the range of 10 to 30 seconds. Pearson's r values close to 1 or –1 indicate strong positive or negative correlation, respectively. Pearson's r values close to zero indicate negligible correlation.



For comparison, the values of NITBUT are plotted against TBUT for NITBUT <10 seconds (Figure 4b). As discussed above, for this range of NITBUT values, most of the TBUT values are smaller than NITBUT values. The Pearson's r coefficient for this range is 0.373, indicating little correlation between the values.



For comparison, the values of NITBUT are plotted against TBUT for NITBUT in the range of 10 to 30 seconds (Figure 4c). For this range of NITBUT values, there does not appear to be a consistent trend of one value being higher than the other. The Pearson's r coefficient for this range is 0.612, indicating some correlation between TBUT and NITBUT.



Several statistical tests were performed to verify if any significant differences exist between the two measurement techniques. These tests are discussed below.

1. t-test for correlated samples:

Student t-test for two correlated samples has been performed and the results are summarized below. The results indicate that the difference in the means is not significant (P=0.205). However, it should be noted that this t-test assumes that both the values are normally distributed. Since we do not know if the two distributions are normally distributed, there is a possibility of violating the underlying assumption. In such a case, it is preferable to do a distribution free test such as Wilcoxon test (see below for results from that test).

t-test for independent samples			
	NITBUT	**TBUT**	**Total**
n	27	27	54
-X	253.12	223.92	477.04
$-X^2$	3236.63	3416.38	6653.01
SS	863.677	1559.34	2438.8
mean	9.3748	8.2933	8.8341
	$Mean_A - Mean_B$	t	df
	1.0815	1.3	26
	P	one-tailed	0.1025
		two-tailed	0.20501

2. Wilcoxon matched pair signed rank test:

This test is more suitable than t-test, if it is not clear if the sample distributions are normally distributed. The primary assumption of the Wilcoxon test is that the matched pairs are drawn randomly and independently, an assumption applicable to our case. The results of the Wilcoxon matched pair signed rank test are given below. For a Z value of 2.28, the level of significance for a non-directional test is slightly more than 0.02 ($P \leq 0.02302$), suggesting that the difference between the two samples is significant.

WILCOXON MATCHED PAIR SIGNED RANK TEST				
N		26		
W		180		
z		2.28		
Level of Significance:				
For a Directional Test				
0.05	0.025	0.01	0.005	0.0005
Non-Directional Test				
--	0.05	0.02	0.01	0.001
$z_{critical}$				
1.645	1.960	2.326	2.576	3.291

Of the 27 subjects, seven were diagnosed with dry eye. Here we will compare the average NITBUT and TBUT values for normal subjects as well as subjects diagnosed with dry eye. The average NITBUT and TBUT values of these two groups of subjects are summarized below. It appears as though the average NITBUT and TBUT values of a group appear to be close and within the standard deviations of the means. However, statistical tests were performed on the data for each of these groups and the significance levels for these tests are also listed in the table.

	Normal subjects		Dry eyed subjects	
	NITBUT	TBUT	NITBUT	TBUT
Average Value	11.636	10.322	5.933	4.237
Std. Dev.	6.289	8.809	1.447	1.498
Significance level for t-test for correlated samples	$P \leq 0.53707$		$P \leq 0.02836$	
Significance level for Wilcoxon matched pair signed rank test	$P \leq 0.1454$		$P \leq 0.03906$	

The Wilcoxon matched pair signed rank test indicates that there is no significant difference between the means of NITBUT and TBUT values for normal subjects, while there appears to be some significant difference between the means of NITBUT and TBUT for dry eyed patients at a significance level of ~0.04 and below.

The results presented in this paper suggest that there appears to be some significant difference ($P \leq 0.02302$) between the tear film stability observed by NITBUT and TBUT methods for all subjects (with and without dry eye). This difference appears to be more prominent for the subjects diagnosed with dry eye ($P \leq 0.03906$). For the normal subjects, there appears to be no significant difference between the means of the NITBUT and TBUT values ($P \leq 0.1454$). It is not clear if the significant difference between the NITBUT and the TBUT is consistent or not for all subjects. For instance, our results suggest the difference between NITBUT and TBUT can be positive or negative for normal subjects. However, the limited results from this study do suggest that NITBUT could be an acceptable alternate test to TBUT and this subject should be investigated further.

REFERENCES :

Brown et al. Mechanical manipulation of the lids and tear break-up time measurements in Hong Kong Chinese. *Ophthal Physiol Opt, v 13, 1993.*

Cho et al. Tear break-up time: clinical procedures and their effects. *Ophthal. Physiol. Opt, v 18 (4), 1998.*

Cho and Douthwaite. The relation between invasive and non-invasive tear break-up time. *Optometry and vision science, v 72 (1), 1995.*

Cho et al. Effect of flourescein on the tear stability of Hong Kong Chinese. *Optometry and vision science, v 73 (1), 1996.*

Patel et al. Effects of flourescein on tear break-up time and on tear thinning time. *Am J Optom Physiol Opt, v 62, 1985.*

Mengher et al. A non-invasive instrument for clinical assessment of the pre-corneal tear film stability. *Current eye research, v 4 (1) 1985.*

Mengher et al. Effect of flourescein instillation on the pre-corneal tear film. *Current eye research, v 4 (1), 1985.*

Thomas et al. Sequential staining: The effect of sodium flourescein, osmolarity, and pH on human corneal epithelium. *Optometry and vision science, v 74 (4), 1997.*