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TEAR VOLUME AND TEAR FILM STABILITY

IN RELATION TO

SOFT CONTACT LENS WEAR IN HONG KONG-CHINESE

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2001
This Thesis is Specially Dedicated

to

My Wife and My Mother
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ABSTRACT

Abstract of thesis entitled: ‘Tear volume and tear film stability in relation to contact lens wear in Hong Kong-Chinese’

Submitted by: Chui Wan Sang

For the degree of: M.Phil.

At the Hong Kong Polytechnic University in May of 2001.

The criteria for a number of clinical tests for the diagnosis of dry eye are well documented. However, practitioners have noted that many contact lens wearers complaining of dryness after commencing lens wear may be free of any signs and symptoms before the commencement of contact lens wear. It seems that conventional tear tests such as the Schirmer test (ST) and the fluorescein tear break-up time (TBUT) test are not sensitive enough to screen out such ‘marginal’ dry eye patients. Modified techniques of tear tests, namely, the non-invasive tear break-up time (NITBUT) test and the cotton thread test (phenol red thread (PRT) and self-prepared cotton thread test (SP-CTT)) now allow non-invasive and less invasive assessments of the tear film stability and tear volume respectively. They have been claimed to be more valid than the conventional versions of these tests and are thus believed to be more sensitive, and able to detect a milder form of dry eye.

Since 1993, Cho and co-workers have performed a series of studies on the tear film stability and tear volume in Chinese eyes. Their results suggested that the NITBUT test and the SP-CTT are reliable in the assessment of tear film stability and tear
volume respectively in Chinese eyes. Cho and Yap (1995) found that soft contact lens wear could cause a transient reduction of NITBUT, but had no effect on SP-CTT value (the PRT was not available in Hong Kong (HK) during the period of their study). When used in isolation, the SP-CTT appeared to be more useful in predicting contact lens wearers who may have symptoms after wearing soft contact lenses while the NITBUT was not. However, due to the small number of subjects in their study, it is necessary to confirm their findings with a larger sample size.

Aims
The current study was therefore designed with the following aims:

a) to investigate the effect of contact lens wear on the tear stability (NITBUT) and tear volume (SP-CTT and PRT values) in a larger group of HK-Chinese (measurements made after removal of contact lens),

b) to determine whether the tests, in isolation or in combination, can be used to predict successful contact lens wear, and if so,

c) to determine the optimal cut-off criterion and the corresponding accuracy of these tests, in isolation or in combination, in predicting successful contact lens wear.

d) to investigate the seasonal change on the test values, and dry eye related problems in contact lens wear.

Methods
Seventy-eight asymptomatic non-contact lens wearers were prescribed with a pair of soft contact lenses and were requested to wear the lenses for 28 weeks on a daily
wear basis. The baseline NITBUT, SP-CTT and PRT values were measured. In 51 of these subjects, the test values were obtained at weeks 2, 9 and 28 after the commencement of soft contact lens wear. After 28 weeks, 15 of these subjects were enrolled into the humidity study, and were given two pairs of new lenses; one pair for use during mid-autumn (dry season) and another pair for use during mid-spring (humid season).

Results and Discussion

We confirmed that the tear volume, as measured by the SP-CTT and PRT test, was not affected by soft contact lens wear. Cho and Yap (1995) reported an initial transient decrease in the NITBUT value at the commencement of soft contact lens wear. However, we did not find any statistically significant change in the NITBUT value (measured after lens removal) after commencement of soft contact lens wear.

After 28 weeks of contact lens wear, the subjects were classified according to their maximum wearing time which did not cause any dry eye-related signs and symptoms. The baseline NITBUT and PRT values were unable to ‘predict’ success of soft contact lens wear. However, the SP-CTT gave a sensitivity and specificity of 81.3% and 55.9% respectively in the prediction of successful contact lens wear when a critical value of 12.5 mm/15 s was used. The positive and the negative predictive values using this cut-off point were 63.4% and 76.0% respectively. We have defined a wetting value by the summation of the NITBUT value and the SP-CTT or the PRT values. However, none of these wetting values were able to provide any useful information on the success of soft contact lens wear.
Seasonal change in Hong Kong did not appear to have a significant impact on the signs and symptoms of dry eye during soft contact lens wear. This was probably because the relative humidity in Hong Kong when the study was conducted was generally high. Also, most of our subjects worked indoors, where the humidity were relatively constant. The result of the main study was therefore unlikely to be affected by seasonal change.
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It is my great pleasure to thank Dr. Pauline Cho, my supervisor. It is impossible to acknowledge adequately for her guidance and support in my study. Dr. Cho has provided invaluable advice during all stages of the methodology, data analysis and thesis writing. She has taught me an invaluable lesson on how to perform a research study. She has also been very kind and patient in improving my skill on paper and thesis writing, and has graciously sacrificed her valuable time in proofreading the manuscript.

A special thank you goes to Dr Brian Brown, my co-supervisor, for his invaluable comments on my thesis writing and proofreading of the manuscript even though he has already left The Hong Kong Polytechnic University.

The research colleagues, Vincent Ng, Peggy Cheung, Camus Choy and Yee Man Kwong, have been a pleasure to work with. Their encouragement and sharing of research experiences are much appreciated. I am also very much in debt to Vincent and Peggy for their assistance in preparation of this thesis.

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## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CTT</td>
<td>Cotton thread test</td>
</tr>
<tr>
<td>CTTw</td>
<td>Cotton thread test using commercially available white cotton thread</td>
</tr>
<tr>
<td>Hirji-Callender grid</td>
<td>Hirji-Callender grid</td>
</tr>
<tr>
<td>NITBUT</td>
<td>Non-invasive tear break-up time</td>
</tr>
<tr>
<td>NITA instrument</td>
<td>Non-invasive tear assessment instrument</td>
</tr>
<tr>
<td>PRT</td>
<td>Phenol red thread using specially designed two-ply thread</td>
</tr>
<tr>
<td>PRT(I)</td>
<td>Phenol red thread using commercially available thread</td>
</tr>
<tr>
<td>SP-CTT</td>
<td>Self prepared-cotton thread test</td>
</tr>
<tr>
<td>ST</td>
<td>Schirmer test</td>
</tr>
<tr>
<td>TBUT</td>
<td>Tear break-up time</td>
</tr>
<tr>
<td>TP-RPT</td>
<td>Tear film pre-rupture phase time</td>
</tr>
<tr>
<td>TTT</td>
<td>Tear thinning time</td>
</tr>
<tr>
<td>Wvp</td>
<td>Wetting value defined by summation of NITBUT and PRT</td>
</tr>
<tr>
<td>Wvs</td>
<td>Wetting value defined by summation of NITBUT and SP-CTT</td>
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Chapter 1

INTRODUCTION

The precorneal tear film plays a unique role in the human eye. It provides a uniform optical surface, flushes away cellular debris and foreign matter and keeps the ocular surface well-lubricated when blinking (Wolff 1946, Duke-Elder and Gloster 1968). It also moisturizes the anterior ocular tissue and nourishes the avascular corneal surface with oxygen dissolved from the ambient air; and also plays a part in the ocular defense system by its antibacterial substances such as lysozyme, beta-lysin and immunoglobulin (Allansmith and Ross 1986; Bron 1988). These functions are especially important in contact lens wear (Guillon and Guillon 1994). Although the interaction between the contact lens and the precorneal tear film is complicated and depends on the lens type (Guillon and Guillon 1994), it is generally accepted that adequate tear volume and stability are essential for successful contact lens wear (Holly 1981a and 1981b, Sharma and Ruckenstein 1985, McMonnies 1990, White 1993).

![Figure 1.1. Structure of the tear film.](image-url)
1.1 Tear physiology

To allow the tear film to achieve its functions, maintenance of the tear film structure is necessary. The following is a brief review of the tear film structure.

The tear film is classically described as a three layered structure (Wolff 1946): a superficial lipid layer, a middle aqueous layer, and an underlying mucous layer which is adjacent to the corneal epithelium (see Figure 1.1). This basic structure has been refined to include the interfaces between the three layers, which are believed to have slightly different mixed composition (Holly and Lemp 1971).

The thickness of the pre-ocular tear film is estimated to be 6 to 9 μm (Mishima 1965, Ehlers 1965a, Maurice 1973), although Prydal and Campbell (1992) suggested that previous reports had vastly underestimated the thickness of the mucous layer, and proposed the tear film thickness to be 40 μm. In a recent study, King-Smith et al. (2000) reported that the tear film was as thin as 3 μm. Since the techniques used by Prydal and Campbell (1992) and King-Smith et al. (2000) are comparatively new, and have not been confirmed by other researchers, in this study, we will adopt the model established and used by many previous investigators. The total tear volume is about 7 μl (Mishima et al. 1966), with the aqueous phase making up the major portion. The tear film is thinnest over the ocular surface between the lid aperture where about 1 μl (Mishima et al. 1966) of the tear fluid is contained. It reduces its thickness from about 7 μm at 5 s after a blink to about 4.5 μm at 30 s after a blink (Ehlers 1965a), while a minimum thickness of 4 μm has been reported (Holly and Lemp 1977). A meniscus is formed along the junction of the lid margin and the globe, which contains approximately 3 μl of tears (Mishima et al. 1966). There is also an unexposed
portion of tear fluid in the fornical space which is approximately 3 μl.

Figure 1.2. A sagittal section through the eyelid showing the locations of the glands responsible for the production of the different components of the tear film.

1.1.1 The tear layers

1.1.1 (i) The superficial lipid layer

The lipid layer is 0.1 μm (Holly and Lemp 1977) thick and contains both polar and non-polar lipids (Nicolaides 1986). Its main function is to retard evaporation of the underlying aqueous fluid. The lipid layer acts as a barrier which aids the stability of the tear film and prevents tears from over-spilling. Lipid is primarily secreted by a series of meibomian glands located in the tarsal plates with openings at the lid margins (see Figure 1.2). The ducts of the meibomian glands are squeezed when blinking so that meibomian material is secreted onto the lid
margin. When the eye opens, an oily film is formed and flows over the aqueous layer. Dysfunction of the ducts or closure of the openings of the glands due to infection or degeneration will cause impairment of the lipid layer and result in reduced tear stability (McCulley and Scialliz 1977) possibly leading to superficial punctate erosions (Brown 1970, Benedetto et al. 1984).

1.1.1 (ii) The aqueous layer

The aqueous layer is approximately 7 μm (Mishima 1965) thick and accounts for 90% of the tear volume. It is secreted by the main and the accessory lacrimal glands of Krause and Wolfring (see Figure 1.2). The aqueous fluid contains ions and molecules including electrolytes, minerals, hydrogen ions, enzymes and proteins. Phagocytes and fragmented epithelial cells are also found there. The electrolytes and mineral ions are responsible for the osmolarity of the tears and play an important role in maintaining epithelial integrity.

The pH of the aqueous tears usually ranges from 7.14 to 7.82 (Carney and Hill 1976). It is lowest on awakening, as a result of an increase in acid by-products associated with the relatively anaerobic conditions of prolonged lid closure. Tear pH increases during waking hours as the eyes are open, allowing loss of CO₂.

Proteins present in the aqueous tears can lower the surface tension of the tear film, thereby allowing it to spread better over the ocular surface. They also play a role in osmotic regulation and act as buffering agents. There are several proteins responsible for ocular defence against microbial invasion (Blades and Craig 1997). They include the bactericidal action of lysozyme and beta-lys, the bacteriostatic action of lactoferrin and the immunologic activity of
immunoglobulins. These defensive actions are maximized with the complement of the active phagocytic cells found in the tear film (Blades and Craig 1997).

The aqueous fluid is secreted as an isotonic or slightly hypotonic solution and flows from the superior temporal to the inferior nasal aspect of the globe. A portion of the basal tear secretion is maintained by the accessory lacrimal glands while the main lacrimal gland is responsible for both the basal and reflex tear secretions (Jordan and Baum 1980). This reflex can be of peripheral sensory origin such as stimulation of the cornea, conjunctiva, skin or nose, or of sensory origin such as retinal stimulation by light or psychogenic stimulation by emotional disturbance (Blades and Craig 1997).

1.1.1 (iii) The mucous layer

The principal sources of mucin are the goblet cells (approximately 1.5 million) on the conjunctival surface (Blades and Craig 1997). The glands of Manz in the limbal ring and the Crypts of Henle in the fornices (see Figure 1.2) also secrete some of the mucin. Other sources are the non-goblet epithelial cells which contain mucous secretory vesicles. Secretions from these vesicles increase in certain ocular conditions such as allergic conjunctivitis. The thickness of the mucous layer is approximately 0.02 to 0.05 μm (Holly 1973), although Prydal and coworkers suggested this to be a gross underestimation, and that it should be greater than 40 μm (Prydal and Campbell 1992, Prydal et al. 1992).

The mucous layer contains mainly carbohydrate and protein, known as glycoprotein. Its main function is to lower the surface tension of the epithelial surface and render it more wettable (Holly and Lemp 1971). Another important
function of this layer is to lubricate the ocular surface, so as to reduce friction during blinking and ocular rotational movements. The mucous layer also protects the epithelial surface against localized surface drying (Holly 1973) and bacterial infiltration by rapidly healing gaps and imperfections (Dilly 1994). Mucous threads are responsible for covering foreign bodies to protect the epithelial surface from abrasion (Norn 1963). The soluble mucus also facilitates tear film stability (Bright and Tighe 1993).

Figure 1.3. The lacrimal drainage system.

1.1.2 Tear dynamics and drainage

The complex sequence of tear dynamics, including the distribution and reformation of the tear film, and the drainage of the tear fluid, is driven solely by the
blinking action. Blinking is controlled by the contraction and relaxation of the orbicular muscles (see Figure 1.2) inside the upper eyelid, and to a certain extent, by the lower eyelid.

When the lids close, the orbicularis muscle contracts. The upper lid moves the larger distance and exerts the greatest force. The lids are pulled nasally, and the temporal side is closed first. The superior and inferior menisci come together and the fornices are compressed. The tear film, together with tear debris, are pushed in an inferior and nasal direction toward the puncta. The contraction of the orbicularis muscle also squeezes the meibomian contents into the tear meniscus.

When the lids open, the aqueous phase of the tears ‘follows’ the upper lid because of surface tension. At the same time, the meibomian content is sheared to form a new lipid layer flowing over the aqueous layer. The shearing force of the lipid layer also helps in dragging up the aqueous layer, because of the difference of surface tension between the aqueous and lipid layers of the tear menisci.

During blinking, the contraction and relaxation of the orbicularis muscle lead to a complex sequence of pressure changes within the drainage system (see Figure 1.3), which drives the tear fluid through it and drains the fluid out into the nasal cavity. The puncta, which are the openings of the tear drainage system through which tears exit, are located at the nasal side of the superior and the inferior lid margins. They are connected to the lacrimal sac through fine ducts called the canaliculi. The sac drains the tear fluid into the nasal cavity through the naso-lacrimal duct. The puncta, the canaliculi, the lacrimal sac and the naso-lacrimal duct comprise the lacrimal drainage system (see Figure 1.3). Reabsorption of tears inside the canaliculi and the tear sac also tend to draw the tear fluid from the
lacrimal lake towards the drainage system, while back flow of tear is prevented by the distal end of the drainage system (see Figure 1.3).

1.2 Tear assessments

A contact lens placed over the ocular surface can be said to be bathing in the tear film. The ability to maintain the integrity of the tear layers is important in successful contact lens wear. The tear film should therefore be carefully assessed prior to and during contact lens wear. Assessments may be qualitative or quantitative. The tear film can also be assessed indirectly, by evaluating the adjacent tissues, such as the corneo-conjunctival epithelium, which may reflect how well the film functions. It is also possible to preview the adequacy of the tear functions through a patient's symptoms using questionnaires. The following paragraphs present brief notes on the assessment techniques used by different investigators.

1.2.1 Tear quantity (tear volume, secretion and production)

Tests for tear quantity are essentially concerned with the aqueous portion of the tear film which is produced by the main and the accessory lacrimal glands and comprises the major portion of the total volume (see Section 1.1.1 (ii)).

Tear volume, quantity, secretion and productions have been used interchangeably by previous investigators.

1.2.1 (i) Schirmer test (ST)

The Schirmer test (ST) was first proposed by Schirmer (1903) for the
measurement of tear production. The bent end of a piece of filter paper strip of 5 mm width is inserted under the lower lid for 5 min, and the length of the wetted portion is measured. A reflex component of the tears is likely to be involved due to the irritation caused by the strip. It can be performed with local anesthetic to measure the ‘basal’ tear production although the existence of ‘basal’ tears has been questioned (Jordan and Baum 1980). It can also be performed in conjunction with stimulation of the nasal mucosa by a cotton wool swab or the retina by strong light. Schirmer test value without local anesthetic, of 15 mm or more in 5 min, is regarded as normal, severe dry eye is indicated when there is less than 5 mm of wetting and mild to moderate dry eye when there is 5 to 10 mm of wetting (Whitcher 1987).

The ST had been criticized as being of poor repeatability (Cho and Yap 1993) and questionable validity (Wright and Meger 1962, Feldman and Wood 1979, Clinch et al. 1983). Cho and Yap (1993) reported that most of the ST values of their normal Chinese subjects were below normal limits or not measurable, and the results obtained were inconsistent. They concluded that the ST is unreliable and of little clinical value in Chinese subjects.

1.2.1 (ii) Cotton thread test (CTT)

In 1975, Kurihashi used a fine white cotton thread instead of the Schirmer strip to measure the tear volume. Observation of the wetted portion required the aid of fluorescein. In 1983, Hamano developed the Phenol red thread (PRT) test in which specially designed fine cotton thread was pre-treated with phenolsulfophthalein dye (see Section 2.1.1 (ii)). The portion of the thread wetted
by the tears changed colour from yellow to red. Cho (1993a) performed a similar test using commercially available orange-red cotton thread (see Section 2.1.1 (iii)). The wetted portion appeared darker and was quite easily observed. The small size of the thread used in the CTT greatly reduced reflex tearing even without the use of local anesthetic. The enhanced absorbency allowed a much shorter testing period which can be as short as 15 s. The CTT was suggested to be a more useful clinical replacement for the ST. Details of these cotton thread tests will be discussed in the next chapter (see Section 2.1).

1.2.1 (iii) Tear meniscus assessment

Assessment of the height and regularity of the tear prism is an easy clinical test for evaluating the volume of the tears. For precise measurement, a graticule can be employed in the slit-lamp eye-piece. An alternative is to compare the tear prism height with the illuminated slit width by setting the slit horizontally in alignment with the lower lid margin, where the rotational knob for the slit width should be calibrated using a microscope scale. Guillon and Guillon (1988) proposed that measurements of the prism height should be made at three locations: below the pupil center, 5mm nasally and 5 mm temporally so that both the tear volume and the regularity of the tear meniscus can be evaluated. However, it has been suggested that the height of the tear prism can be affected by factors other than tear quantity, such as anatomy of the lid (Lambert et al. 1979). Port and Asaria (1990) used an optical pachometer to measure the tear meniscus height. However, this method of measuring the tear meniscus height is not practical in private practices.
1.2.1 (iv) Protein Assay

Lacrimal gland function can also be evaluated indirectly by assessing the tear protein concentration. Reduction of tear secretion can be reflected by the concentrations of lysozyme and lactoferrin, two major lacrimal gland proteins. They can be measured by immunodiffusion assay (Craig and Blades 1997). It has been found that the concentrations of these proteins in dry eye patients were around a tenth of the normal. Lactoplate is a commercially available test for measurement of the lactoferrin concentration. A small filter paper disc is placed in the patient’s inferior fornix for several minutes. After saturation with tears, the disc is placed on immunoreactive gel for three days. The sizes of the precipitation rings which form on the gel are proportional to the concentration of lactoferrin in the tear sample. Since this test is expensive and time consuming, it is mainly used for research purposes.

1.2.1 (v) others

There are some tests designed for measurement of the turnover rate of the tears such as, the Dilution test (Norn 1965, Xu et al. 1995), Gamma scintigraphy (Rossomondo et al. 1972) and Fluorophotometry (Mishima et al. 1966). However, these tests have to be performed in a laboratory.

1.2.2 Tear quality

Clinically, tear quality is usually assessed by assessing the tear stability, while some laboratory tests are used in experimental studies.
1.2.2 (i) Fluorescein tear break-up time (TBUT) test

This test involves the instillation of fluorescein into the tear film and observation for change of the dyed tear film with the aid of a slit-lamp, with a broad-beam of cobalt blue light (Norn 1969). Localized discontinuities of fluorescein observation are believed to correspond to the rupture of the tear film. The time between a complete blink to the first appearance of a 'dark spot' is recorded as the tear break-up time (TBUT). Reports of the TBUT test are well documented in the literature although the results are inconclusive and contradictory (Vanley et al. 1977, Tonge et al. 1991). The main criticism is that the instillation of fluorescein may affect the stability of the tear film (Mengher et al. 1985b, Norn 1986, Holly 1987, Tonge et al. 1991)

1.2.2 (ii) Non-invasive tests of tear film stability

The invasive nature of the TBUT test has lead to the development of non-invasive tear assessment techniques. These tests depend on the observation of interference patterns of the pre-corneal tear film (Guillon 1986a), or the distortions of a reflected image on the corneal surface (Mengher et al. 1985a and 1986, Hirji et al. 1989, Tonge et al. 1991, Cho 1993, Madden et al. 1994).

The former, referred to as lipid interferometry, can be assessed with the aid of a slit-lamp. It can be performed by two methods. The first one is a narrow-field specular reflection method where the bright reflection from a slit beam is observed at high magnification (30 – 40X) (Marx 1921 cited by Guillon and Guillon 1994, Ehlers 1965b). The limited field of view and the risk of artificially drying the tear film due to the light source have resulted in a preference for the second method
using the Tearscope. The Tearscope is a hand held instrument specially designed to observe the tear film (Guillon 1986b). It consists of a white hemispheric cup illuminated by a cold cathode ring for projection of the specular reflection on the cornea, and can be used in conjunction with the magnification system of a slit-lamp. The illumination of the cold cathode ring of the Tearscope avoids the use of the light source of the slit-lamp during observation. The interference pattern on the tear film is essentially caused by the nature of the thin lipid layer. Different interference patterns had been observed. The appearance and the changes of the interference patterns can reflect the thickness and stability of the tear film (Guillon 1986). Details of the structure and applications of the Tearscope will be discussed in Section 2.2.1 (iv).

The technique which involves the observation of corneal reflected image is commonly termed the non-invasive tear break-up time (NITBUT) test (Mengher et al. 1985a, Tonge et al. 1991, Cho 1993, Cho and Brown 1993). The time from a complete blink to the first appearance of a discontinuity or distortion is recorded as the NITBUT. Some investigators have observed the reflected image of the mires of a Bausch & Lomb Keratometer and called this measurement the tear thinning time (TTT) (Patel et al. 1985, Little and Bruce 1994). Details of the NITBUT tests will be discussed in the next chapter (see Section 2.2).

The main advantage of such non-invasive techniques is that no fluorescein instillation is required. This allows a more natural observation of the tear stability, and does not affect the results of other tear tests following this test.
1.2.2 (iii) Tear osmolality

Tear osmolality has been shown to increase in dry eye, and this was thought to play a role in the ocular damage associated with dry eye (Guillon and Guillon 1988). It can be measured by the freezing point depression nanolitre osmometry (Benjamin and Hill 1983). A tear sample is collected from the inferior tear meniscus using a fine glass capillary tube. Nanolitre volumes of sample are placed into wells on the cooling module of an osmometer. The samples are frozen rapidly and the temperature increased slowly until the last crystal of each sample melts. The osmolality of the sample can be calculated since it is proportional to its freezing point. However, this technique is restricted to laboratory studies while attempts are still being made to design an osmometer for clinical use.

1.2.2 (iv) Tear evaporation

The rate of tear evaporation can indirectly indicate the lipid layer integrity (Refojo et al. 1986, Craig and Blades 1997). It is proportional to the vapour pressure gradient in front of the ocular surface. This gradient can be measured by a pair of sensors attached in a pair of modified swimming goggles (Refojo et al. 1986). Knowing the distance between the sensors and their distance from the ocular surface, the vapour pressure gradient can be calculated. Vapour contributed by the skin should be taken into account by comparing the result measured with the eyes open and with the eyes close respectively. The rate of tear evaporation was calculated using these data and the ambient environmental conditions. To date, this technique is still restricted to the laboratory.
1.2.2 (v) Tear ferning test

In this test, a tear sample is placed on a slide and allowed to air dry (Rolando et al. 1986) or refrigerated at 4°C (Norn 1988). A ‘fernig’ pattern, resulting from crystallization of the tear constituents, can be observed when the sample is viewed under a microscope. The extent of ferning can be graded. The test was believed to be a quantitative measure of mucus in the tear sample (Norn 1988) but other studies suggested that it may reflect the tear protein profile (Kogbe and Liotet 1987). Contamination of the sample, by meibomian secretion or cosmetics (Norn 1988), had been suggested to be possible sources of errors.

1.2.3 Tests related to the adjacent tissues of the tear film

Evaluation of adjacent tissues may provide some insights into the quantitative and qualitative aspects of the tear film. There are tests available for the assessment of the meibomian glands and the conjunctival and corneal epithelial surfaces.

1.2.3 (i) Meibomian gland expression

Expression of the meibomian gland by digital compression on the lower lid allows assessment of the quality and quantity of the tear lipid. In normal cases, the meibomian secretion should be expressed easily as a clear fluid. However, dysfunction of the meibomian glands may result in cloudy or toothpaste-like output, or even in the absence of expression due to blockage of the glands. The severity of the problem is graded according to the clearness of expression and the number of blocked orifices (Guillon and Guillon 1994).
1.2.3 (ii) Impression cytology

Impression cytology allows the assessment of the epithelial morphology (Nelson et al. 1983) and goblet cell density (Nelson and Wright 1986) of the conjunctiva. It involves pressing a piece of Millipore filter paper onto the bulbar or tarsal conjunctiva. A fine layer of superficial conjunctival epithelial cell tissue adheres to the filter when it is removed. This tissue can be fixed and stained, and the cells can be observed under an electron microscope. Squamous metaplasia of the conjunctival epithelium and reduction of goblet cells density are usually found in dry eye patients and is related to the severity of the condition.

1.2.3 (iii) Ocular surface observation

Interpalpebral conjunctival and corneal staining are commonly seen in dry eye. A variety of dyes are employed in ophthalmic work but, in clinical practice, the most common ones are sodium fluorescein and rose bengal.

Fluorescein 'stains' the lesion by accumulation of the dye at the intercellular spaces without staining the cells (Feenstra and Tseng 1992), and is best observed with cobalt blue light. The dye is commonly instilled by wetting a commercially available fluorescein sodium strip with a drop of saline, and applying the strip to the tear film overlying the inferior bulbar conjunctiva. Observation relying on a single application of the dye may lead to variable results (Caffery and Josephson 1991).

Rose bengal stains degenerated and devitalized epithelial cells (van Bijsterveld 1969). The dye can be instilled by wetting a commercially available rose bengal-impregnated strip with a drop of saline and applying it to the eye in the same
manner as with the fluorescein strip. Staining may appear early in most patients with incipient dry eye, yet some normal eyes may stain minimally (Baum 1985). The drop is quite irritating to the eye especially in dry eye patients and hence, it is less often used in contact lens practice.

Grading or scoring systems, for the staining observed, are available for more repeatable measurements which allow evaluation of severity and changes of the condition (van Bijsterveld 1969, LaRoche and Campbell 1988). Critical issues on these techniques include: how long after instillation should the grading be done, should drawings or photos be used, and what grading system should be used (Lemp 1995).

1.2.4 Dry eye questionnaire

The patient's history and symptoms have been found to be good diagnostic tools in identifying patients with tear deficiency (McMonnies 1986). Symptoms can be graded by a questionnaire, such as The Dry Eye Questionnaire of McMonnies (1986) (see Appendix I). It is composed of questions on previous diagnosis of dry eye, primary symptoms and their frequency, secondary (provoked) symptoms and systemic and ocular conditions associated with dry eye. A marking scheme has been devised to ascertain the severity of symptoms. Sensitivity and specificity of 85% and 88% respectively have been found in the diagnosis of 50 patients with Sjögren's syndrome and 124 normal subjects (McMonnies et al. 1998).
1.3 Comment

A variety of tests are available for the assessment of the tear film and its functions. However, many of them are expensive and time consuming or are restricted to laboratory or experimental use. The most common tests used for assessing tear quantity and quality in private practice are still the ST and the TBUT test although the CTT and NITBUT test have been claimed to be superior replacements of these two tests. The next chapter is a more detailed review of the literature on the CTT and the NITBUT test, as these two tests will be used in the experimental studies reported in this thesis.
2.1 Cotton thread test (CTT)

The ST has been used to assess the tear volume for almost a century, despite the fact that the test was widely criticised as a crude test of poor reliability (Henderson and Prough 1950, Wright and Meger 1962, Pinschmidt 1970, Feldman and Wood 1979, Cho and Yap 1993). The shortcomings of the test have led to the development of the CTT where a piece of cotton thread is used instead of a strip of filter paper. The procedure for CTT is similar to that for the ST and the testing period can be as short as 15 s. The small size of the thread and the short testing time render the test much less invasive and offer more advantages when compared to the ST.

2.1.1 Development of different cotton thread tests

2.1.1 (i) Cotton thread test using commercially available white cotton thread (CTTw)

Kurihashi et al. (1975) first suggested the use of a cotton thread (#40/2) to measure the tear volume of human subjects. Kurihashi (1982) later developed the
test using commercially available white cotton thread (#82/3) of better quality, made of America pima cotton (Yokota Co.). The thread was cut into lengths of about 60 mm with one end of about 5 mm bent to form a hook. To perform the test, the bent end was hooked under the lower lid for 5 to 30 seconds (see Section 2.1.3 (ii)), and the length of the wetted portion was measured. The boundary of the wetted portion can be observed with the aid of fluorescein. Kurihashi (1986) described three possible methods of applying this test. In Method 1, the bent end was dyed with 10% fluorescein prior to insertion into the conjunctival sac so that the wetted portion will turn yellow. In Method 2, before inserting the thread, the lower palpebral conjunctival sac of the tested eye was stained with fluorescein. The white cotton thread soaked up the stained tears during measurement. In Method 3, the portion around the wetted boundary of the white cotton thread was blotted with fluorescein paper after removal from the conjunctival sac so that only the wetted portion at the boundary of the thread was stained yellow. Kurihashi (1986) suggested that Method 3 was superior to the other two methods since no prior staining was necessary and the test could be administered to soft contact lens wearers. Also, wet lengths of <5 mm, as in severe lacrimal deficiency, can be measured. In this thesis, we will refer to this test as CTTw.

Hamano et al. (1983) made a number of comments on Method 1 of the CTTw:

a) it was difficult to limit the fluorescein to exactly 5 mm of the thread,

b) fluorescein did not function well as an indicator of wetting, since in some cases, the wetted portion of the thread did not change color,

c) the fluorescein occasionally diffused into the tears, which created a problem
for soft lens wearers, and may have stimulated reflex tearing in some patients.

2.1.1 (ii) The Phenol red thread (PRT) test

Hamano et al. (1983) modified the CTT by impregnating commercially available white cotton thread with phenol red dye (phenosulfophthalein). This thread changes color from yellow to red over a pH ranged from 6.6 to 8.2. The portion of the thread wetted by tears was therefore easily observed. The absorbency was greater than that of the white cotton thread used by Kurihashi (1986). They used these phenol red threads in a series of preliminary studies. Hamano and co-workers later suggested that commercially available sewing thread contained a number of additives and impurities. So, they designed a high quality two-ply cotton thread, with a diameter of approximately 0.2 to 0.3 mm, specifically for their purpose and the test was called the Phenol red thread (PRT) test. A series of experimental studies showed enhanced absorbency and improved reproducibility of results with the new thread. They also suggested a testing period of 15 s as it was sufficient to differentiate between normal and possible dry eye patients (see section 2.1.3(ii)).

Blades and Patel (1996) prepared their own PRT test using commercially available white cotton thread to investigate the dynamics of tear flow within the thread.

To avoid confusion, the acronyms PRT(I) will be used to refer to phenol red thread test which uses commercially available thread, and PRT will be used to refer to the phenol red thread test which uses the specially designed two-ply thread.
2.1.1 (iii) The Self-Prepared Cotton Thread Test (SP-CTT)

Cho and co-workers (Cho 1993a, Cho and Yap 1994, Cho and Douthwaite 1994, Cho and Kwong 1996, Kwong and Cho 1998) used treated commercially available colored cotton thread (about 0.2 mm thick) to conduct a series of studies investigating the tear volume of Hong Kong (HK)-Chinese, as the PRT test was not available at the time of their studies. Cho (1993a) compared cotton threads of different brands and colors to select the thread which showed the best performance in absorbency and ease of observation of the wet length. Having decided on the type of thread to use, the chosen thread was boiled in water to remove the wax and to loosen the thread for better absorbency, air-dried and sterilized by soaking in 90% alcohol before air drying again. The treated thread was cut into short lengths of about 70 mm and stored in sterile plastic bags for later use. The wet length was not difficult to observe since the wetted portion appeared darker. This test was later referred to as the Self-Prepared Cotton Thread Test (SP-CTT) (Kwong and Cho 1998) to differentiate it from other CTT.

2.1.2 Pros and Cons of the CTT

Advantages:

The CTT has been claimed to have several advantages over the ST in measuring tear volume:

1. the test is quick and easy to perform (Hamano et al. 1983),

2. it produces minimal discomfort, and thus the chance of reflex tearing is reduced (Hamano et al. 1983),

3. the short testing period can minimize effects of environmental factors such as
humidity (Hamano et al. 1983),

4. the result obtained is statistically more reliable (Cho 1993a, Cho and Kwong 1996),

5. it is sensitive to small variations of tear fluid (Hamano et al. 1983, Blades and Patel 1996),

6. the small amount of tear fluid uptake allows successive measurements (Kurihashi 1986) or performance of other tear tests (such as NITBUT) with a short resting period between tests (Cho et al. 1996a),

7. the small size of the thread is unlikely to cause apprehension to patients especially for small children (Kurihashi et al. 1977, Cho 1993a, Cho and Yap 1994).

Disadvantages:

Despite the numerous advantages over the ST, there are a few criticisms of the CTT:

1. the relatively low absorption capacity of the thread makes it possible that some individuals may secrete tears at a higher rate than can be absorbed by the thread (Lupelli 1988),

2. the test may only be measuring the residual tears in the cul-de-sac rather than the tear secretion (Lamberts 1979).
Table 2.1 Summary of results of SP-CTT/PRT test obtained by previous investigators.

<table>
<thead>
<tr>
<th>Investigator(s) (Year)</th>
<th>Type of thread</th>
<th>Testing period (s)</th>
<th>No. of subjects (eyes)</th>
<th>Location of insertion</th>
<th>Mean(SD) (mm/ testing period)/ Result</th>
<th>Eye posture</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kurihashi et al. (1977)</td>
<td>CTTw (M1) 3x on each eye</td>
<td>5, 10, 30</td>
<td>56 (112) 49 (98) 50 (100) (Presumably Japanese)</td>
<td>Upper temporal</td>
<td>RE: 19; LE: 18 RE: 19; LE: 19 RE: 22; LE: 22</td>
<td>Closed</td>
<td>Entire wet length</td>
</tr>
<tr>
<td>Hamano et al. (1983)</td>
<td>PRT (i)</td>
<td>5, 10, 15, 20, 30, 60, 120</td>
<td>8</td>
<td>Inferior temporal</td>
<td>5-25 at 30 s</td>
<td>Closed</td>
<td>Entire wet length</td>
</tr>
<tr>
<td></td>
<td>PRT (i) + LA</td>
<td>5, 10, 30, 60, 120</td>
<td>6 (12) (6) RE (6) LE (control eye)</td>
<td>Inferior temporal</td>
<td>No effect of LA on wet length</td>
<td>No significant difference in wet length between the three locations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRT (i) + saline</td>
<td>?</td>
<td>6 (12)</td>
<td>Inferior temporal: nasal, central, temporal</td>
<td>Non CLW: 16.7 CLW: 16.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PRT</td>
<td>15</td>
<td>1890 (3780) non CLW (3336) CLW (Presumably Japanese)</td>
<td>Inferior temporal</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key:
- SD - standard deviation
- CTTw - (white) cotton thread test
- PRT (i) - Phenol red thread prepared by using commercially-available cotton thread dyed with phenol red dye
- PRT - Phenol red thread
- CLW - contact lens (soft, PMMA or RGP) wearers
- LA - Local anaesthetic
- ? - not specified or not clear
- M1, M2, M3 - different test procedures used by Kurihashi (see text)
- M? - test procedure (Kurihashi's) not stated (data collected from patient's records from different clinics from 1984-1987)
Table 2.1 Summary of results of SP-CTT/PRT test obtained by previous investigators (continued).

<table>
<thead>
<tr>
<th>Investigator(s) (Year)</th>
<th>Type of thread</th>
<th>Testing period (s)</th>
<th>No. of subjects (eyes)</th>
<th>Location of insertion</th>
<th>Mean(SD) (mm/ testing period)/ Result</th>
<th>Eye posture</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kurihashi (1986)</td>
<td>CTTw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1: 3x on each eye</td>
<td>3-7</td>
<td>(100)</td>
<td>?</td>
<td>1st: 22.5(5.9); 2nd: 21.0(5.9); 3rd: 21.4(6.3); overall: 22.5(7.5)</td>
<td>Closed</td>
<td>Entire wet length</td>
</tr>
<tr>
<td></td>
<td>M1: 3x on each eye</td>
<td>3-7</td>
<td>(3000)</td>
<td>Inferior</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2</td>
<td>5-7</td>
<td>62 (124)</td>
<td>Inferior</td>
<td>24.9(7.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M3</td>
<td>5-7</td>
<td>105 (210)</td>
<td>Inferior</td>
<td>21.0(6.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M2 2x on each eye</td>
<td>5</td>
<td>105 (210)</td>
<td>1st Inferior</td>
<td>23.3(7.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>5 s</td>
<td>(112) (Presumably Japanese)</td>
<td>Inferior 2nd Inferior</td>
<td>25.7(8.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Inferior</td>
<td>25.9(10.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamano et al. (1990)</td>
<td>PRT</td>
<td>15</td>
<td>(11336) CLW (Presumably Japanese)</td>
<td>Inferior temporal</td>
<td>Wet length decreases with increasing age</td>
<td>Opened</td>
<td>Entire wet length</td>
</tr>
<tr>
<td>Cho (1993a)</td>
<td>SP-CTT</td>
<td>60</td>
<td>54 HK-Chinese</td>
<td>Inferior temporal</td>
<td>22.8(9.4)</td>
<td>Closed</td>
<td>From the bend</td>
</tr>
</tbody>
</table>

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- PRT: Phenol red thread
- CLW: contact lens (soft, PMMA or RGP) wearers
- SP-CTT: Self prepared-cotton thread test
- LA: Local anaesthetic
- ?: not specified or not clear
- M1, M2, M3: different test procedures used by Kurihashi (see text)
- M?: test procedure (Kurihashi's) not stated (data collected from patient's records from different clinics from 1984-1987)
Table 2.1 Summary of results of SP-CTT/PRT test obtained by previous investigators (continued).

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<thead>
<tr>
<th>Investigator(s) (Year)</th>
<th>Type of thread</th>
<th>Testing period (s)</th>
<th>No. of subjects (eyes)</th>
<th>Location of insertion</th>
<th>Mean(SD) (mm/ testing period)/ Result</th>
<th>Eye posture</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sakamoto et al. (1993)</td>
<td>PRT</td>
<td>15</td>
<td>US-Caucasians: 250M (500) 250F (500) Total 500 (10000) Japanese: 250M (500) 250F (500) Total 500 (1000)</td>
<td>Inferior temporal</td>
<td>25.0(9.4) 22.8(9.5) 23.9(9.5) 19.8(8.6) 17.7(8.5) 18.8(8.6)</td>
<td>Opened</td>
<td>Entire wet length</td>
</tr>
<tr>
<td>Cho &amp; Yap (1994)</td>
<td>SP-CTT</td>
<td>60</td>
<td>91 HK-Chinese 35 S-Chinese</td>
<td>Inferior temporal</td>
<td>17.5(8.1) 19.6(12) Pooled: 18.1(9.3)</td>
<td>Closed</td>
<td>From the bent</td>
</tr>
<tr>
<td>Cho &amp; Douthwaite (1994)</td>
<td>SP-CTT</td>
<td>60</td>
<td>15 HK-Chinese 13 UK-Caucasians 21 HK-Chinese</td>
<td>Inferior temporal</td>
<td>22.5(10.7) 25.8(9.7) 22.7(9.5) Return to baseline in 10 min after a CTT measurement</td>
<td>Closed</td>
<td>From the bent</td>
</tr>
</tbody>
</table>

Key:
- SD - standard deviation
- CTTw - (white) cotton thread test
- PRT (f) - Phenol red thread prepared by using commercially-available cotton thread dyed with phenol red dye
- PRT - Phenol red thread
- CLW - contact lens (soft, PMMA or RGP) wearers
- SP-CTT - Self prepared-cotton thread test
- LA - Local anaesthetic
- ? - not specified or not clear
- M1, M2, M3 - different test procedures used by Kurihashi (see text)
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- M, F - male, female subjects respectively
Table 2.1 Summary of results of SP-CTT/PRT test obtained by previous investigators (continued).

<table>
<thead>
<tr>
<th>Investigator(s) (Year)</th>
<th>Type of thread</th>
<th>Testing period (s)</th>
<th>No. of subjects (eyes)</th>
<th>Location of insertion</th>
<th>Mean(SD) (mm/ testing period)/ Result</th>
<th>Eye posture</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Little &amp; Bruce (1994)</td>
<td>PRT (on 2 consecutive days)</td>
<td>15</td>
<td>17 Caucasians</td>
<td>Inferior temporal</td>
<td>2 consecutive days: 27(8) on day one 27(7) on day two</td>
<td>Opened</td>
<td>Entire wet length</td>
</tr>
<tr>
<td>Cho &amp; Kwong (1996)</td>
<td>SP-CTT PRT</td>
<td>15</td>
<td>15 HK-Chinese</td>
<td>Inferior temporal</td>
<td>CTT: 11.8(4.4) PRT: 15.4(4.9)</td>
<td>Closed</td>
<td>From the bend</td>
</tr>
<tr>
<td>Blades &amp; Patel (1996)</td>
<td>PRT (I)</td>
<td>15, 30, 45, 60, 75, 90, 105, 120</td>
<td>40 (14M, 26F) (presumably Caucasians)</td>
<td>Inferior temporal</td>
<td>Wetting of PRT is not related to gender and age</td>
<td>?</td>
<td>Entire wet length</td>
</tr>
<tr>
<td>Kwong &amp; Cho (1998)</td>
<td>SP-CTT PRT</td>
<td>15</td>
<td>46 HK-Chinese (Dry eye) 37 HK-Chinese (Normal eye)</td>
<td>Inferior temporal</td>
<td>SP-CTT: Dry eye - 6.2(4.2) Normal eye - 8.3(4.3) PRT: Dry eye - 10.7(4.5) Normal eye - 11.4(4.5)</td>
<td>Opened</td>
<td>Entire wet length</td>
</tr>
</tbody>
</table>

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M, F - male, female subjects respectively
2.1.3 Factors affecting the CTT

In view of the small size and the good absorbency of the cotton thread, the CTT result may be easily affected by the testing method, location of insertion, testing period, open or close eye during the test, or consecutive measurements. Table 2.1 presents a summary of the investigations and results reported by previous investigators.

2.1.3 (i) CTT measurement

The test value of the CTT may depend on the location of insertion, testing procedure and how the wet length is measured after the thread is removed.

Hamano et al. (1983) compared the wet length obtained from the superior and inferior conjunctival sacs (temporal side) of 10 eyes. Longer wet lengths were obtained from the superior conjunctival sacs and they attributed the result to the anatomy of the tear secretion mechanism. Measurements made with the thread located temporally, centrally and nasally in the inferior conjunctival sacs on the right and left eyes of six subjects were also compared but no significant difference was found. However, in the statistical analysis of their data, they treated data obtained from the right and left eyes of a subject as independent data values and so the validity of their results may be questionable on statistical grounds (see Section 2.1.6). They suggested inserting the thread in the inferior temporal position when performing the thread test as the thread was relatively easy to insert in this location.

Kurihashi (1986) also reported a longer wet length from the superior conjunctival sac in both eyes of 105 normal subjects. Kurihashi (1986) reported that the wet
length of CTTw measured by Method 2 (see Section 2.1.1 (i)) were significantly greater than those for Method 1 while results obtained from both Methods 1 and 2 were greater when compared with Method 3. The different results obtained may be due to the use of fluorescein in Methods 1 and 2. Also, they used both right and left eyes as independent data points (see Section 2.1.6).

In the majority of the studies on the CTT, the entire length of the wetted portion was measured (see Table 2.1). But in several of the studies of Cho and coworkers (Cho 1993a, Cho and Yap 1994, Cho and Kwong 1996) using the SP-CTT (see Section 2.1.1 (iii)), the wet length was measured from the bent end. Some investigators required their subjects to close their eyes during measurement while other investigators instructed the subjects to blink normally (Hamano et al. 1990, Sakamoto et al. 1993, Little and Bruce 1994, Kwong and Cho 1998) (see Table 2.1).

2.1.3 (ii) Testing periods

Kurihashi et al. (1977) performed CTTw (using Method 1) on three groups of subjects (n=56, 49 and 50). Testing periods of 5 s, 10 s and 30 s were used on the groups respectively. No significant difference was found between groups. But they commented that intra-subject variation increased with longer testing periods and repeated measurements.

Hamano et al. (1983) performed PRT(I) test (see Section 2.1.1 (ii)) on eight normal subjects. They found that the wet length reached a steady state after 30 s in most eyes. They also reported another study where the PRT test was performed on 455 normal and 76 dry eye subjects and testing periods of 5-60 s were used. They
defined 'dry eye' as eyes showing <6 mm of wet length in 15 s. Hamano and co-
workers observed that the distribution curves for wet length for different testing
periods were similar for normal and dry eye subjects, with the dry eyes
consistently showing lower wet lengths. They suggested that a 15 s testing period
was sufficient for differentiation between normal and dry eye patients, and that a
longer testing period may be valuable to calibrate the degree of dryness.

2.1.3 (iii) Consecutive measurements

Hamano et al. (1983) suggested that the small amount of tears required for the
PRT test allows the test to be repeated several times at a relatively short interval
(1-5 min).

Kurihashi (1986) reported no significant difference between three consecutive
measurements obtained from 100 normal eyes when using Method 1 of their
CTTw (see Section 2.1.1 (i)) and testing periods of 3 to 7 s.

Cho and Douthwaite (1994) performed the SP-CTT on 15 HK-Chinese and 13 UK
Caucasian subjects using a testing period of 1 min, at 5, 10 and 15 min intervals.
They found that the SP-CTT value obtained 5 min after the first SP-CTT was
significantly reduced compared to the baseline value. They found no significant
difference between the baseline value and SP-CTT values obtained at the 10 and
15 min intervals. They concluded that 10 min was a sufficient time for tear
recovery after a SP-CTT using a testing period of 1 min.

2.1.3 (iv) Local anaesthetic

Hamano et al. (1983) observed the effect of instillation of a local anaesthetic to
the tear volume as measured by their PRT(I) test. A drop of anaesthetic (not specified) and normal saline were added to the right and left eyes of the subject respectively. The wet length markedly increased initially and the 'pre-drop' level was re-established after five minutes. However, they did not state the number of eyes studied. In the same paper, they continued to report an experiment where Oxybuprocaine (0.4%) was instilled into the right eyes of six subjects and 5 min later, the PRT(I) test was preformed three times in both eyes, at 1 min interval. The left eyes served as controls. No significant difference in the wet lengths between the right and left eyes was found. They concluded that 'the thread does not produce sufficient reflex tear secretion in the short measurement period to require the use of an anaesthetic'.

2.1.3 (v) Age

A number of investigators have reported the effect of age on tear volume but there is a lack of agreement on the relationship between age and CTT value. Kurihashi (1986) reported a study where the CTTw (Method 1 - see Section 2.1.1 (i)) was used on 3000 normal eyes (1088 male, 1912 female), and wet lengths were significantly greater in subjects of age below 19 and above 80 years when compared to subjects of age 20 to 79 years. He attributed the result to apprehension of the test in the younger subjects, and reduced tear drainage in the older subjects. It appeared that he was treating the measurements from the two eyes as independent data points (see Section 2.1.6). Hamano et al. (1990) classified 11,366 eyes of contact lens wearers aged from 10 to 59 years according to the lens types and the age of the subjects. They found that
the percentage of eyes with PRT value ≥9 mm/15 s increased with increasing age and the percentage of eyes with PRT value ≥15 mm/15 s decreased among progressively older groups. Sakamoto et al. (1993) compared the PRT values of 500 Japanese and Americans of age 0 to >60 years. They found no significant correlations between age and PRT values in either group of subjects. They also used both eyes of each subject and treated them as independent data.

On the other hand, Cho and Yap (1994) found significant correlations between age and SP-CTT value in their Chinese subjects - 91 HK-Chinese of age 8 to 70 years (45 male, 16 female) and 35 Singapore (S)-Chinese of age 7 to 65 years (14 male, 21 female).

Blades and Patel (1996) measured the PRT(I) of the right eyes of 40 normal subjects aged 18 to 86 years (14 male, 26 female), and reported differences in the wet value between age groups only at the testing periods of 30, 60, and 90 s but not at 15, 45, 75, 105 and 120 s. They concluded that the wetting of PRT is not related to age. They suggested that the replenishment of the lacrimal lake may be somewhat delayed in the older population due to the reduction of the output capacity and corneal sensitivity, and this produced the differences they observed.

2.1.3 (vi) Gender

Most studies have suggested that the CTT values of females are lower but some have reported no significant differences between males and females.

Kurihashi (1986) reported that the mean wet length of female subjects measured by their CTTw (Method 1 - see Section 2.1.1 (i)) were lower than those of male subjects for subjects aged 0 to 89 years, except for those in the age range of 20 to
49 years. However, they used both right and left eyes as independent data points (see Section 2.1.6).

Sakamoto et al. (1993) found significantly lower PRT values in females in both Japanese and American subjects. The mean±SD PRT values for male and female Japanese subjects were 19.8±8.6 mm/15 s and 17.7±8.5 mm/15 s respectively, and for the American subjects, the values were 25.0±9.4 mm/15 s and 22.8±9.5 mm/15 s respectively. They also used both right and left eyes as independent data points (see Section 2.1.6).

Cho and Yap (1994) found no significant difference in the SP-CTT value between their male and female HK- and S-Chinese subjects. The mean±SD SP-CTT values for male and female HK-Chinese were 18.3±9.0 mm/15 s and 16.7±7.0 mm/15 s respectively, and for S-Chinese, they were 15.9±12.1 mm/15 s and 22.1±11.5 mm/15 s respectively.

Blades and Patel (1996), in a study of the dynamics of wetting within a PRT(I) thread, found no differences in the wetting characteristic between male and female subjects and concluded that there were no gender difference in the tear production rate.

2.1.3 (vii) Humidity

Effect of ambient conditions on CTT values are expected to be minimal as the testing period used is short.

In an in-vitro study, Hamano et al. (1983) investigated the effect of relative humidity on the wet length of the PRT(I) test. The wet lengths of 20 suspended threads were measured after 15 s, at an ambient temperature of 25°C and relative
humidities of 35%, 60% and 80%. The results were not significantly affected by the different humidities.

Sakamoto et al. (1993) also investigated the effect of different ambient temperatures (United States: 20-29°C, Japan: 18-33°C) and humidities (United States: 42-81%, Japan: 27-84%) on the PRT values on 1000 Japanese and American eyes (see Section 2.1.6). They reported no significant effect of temperature and humidity on PRT values.

2.1.3 (viii) Racial difference

It appears that the Oriental population has a lower CTT wet length than Caucasians.

Sakamoto et al. (1993) compared the PRT values of 500 Japanese (1000 eyes) and 500 Americans (1000 eyes) and found that the mean wet length of the American subjects was significantly longer than that of the Japanese subjects. The mean±SD wet lengths of the Americans and the Japanese were 23.9±9.5 mm/15 s and 18.8±8.6 mm/15 s respectively. However, since they used both right and left eyes as independent data points, their results may be questionable on statistical grounds as the variability was probably underestimated. (see Section 2.1.6).

Cho and Douthwaite (1994) found that the mean SP-CTT value of Chinese eyes was significantly lower than that of the UK-Caucasian. The mean±SD SP-CTT values obtained from 15 HK-Chinese and 13 UK-Caucasians were found to be 22.5±10.7 mm/60 s and 25.8±9.7 mm/60 s respectively.
2.1.3 (ix) Contact lens wear

Although it has been reported that tear volume may decrease after commencing contact lens wear, the CTT value has been found to be unaffected by contact lens wear.

Hamano et al. (1983) compared the PRT value of a group of contact lens wearers (3336 eyes) and a group of non-contact lens wearers (3780 eyes). They found no significant difference in the PRT value between the two groups (see Section 2.1.6).

Cho and Yap (1995) monitored changes in the tear volume with soft contact lens wear in 35 HK-Chinese by measuring the SP-CTT values before and after different periods of lens wear. They did not find any significant change in the SP-CTT value after 2, 9 and 28 weeks of lens wear.

2.1.4 Reliability

Most investigators have found the CTT to be reliable.

Kurihashi (1986) reported the results of 54 measurements of CTTw (Method 1 - see Section 2.1.1 (i)) obtained from a 44 year-old female subject with right epiphoria due to dysfunction of the lacrimal drainage, on 18 occasions. The abnormal eye consistently showed higher wetting than the normal eye. He commented that the result demonstrated good reproducibility of the test. He also reported a case in which a 60 year-old women suffering from Sjogren's syndrome was tested 16 times during 14 months using CTTw, Method 1. Each test consisted of three successive measurements. The dryer eye consistently showed shorter wet lengths than the fellow eye.

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Cho (1993a) tested the repeatability of the SP-CTT by performing the test eight times on the left eye of seven normal HK-Chinese on separate days over a period of two weeks. No significant difference in the SP-CTT values taken on different days was found.

Little and Bruce (1994) performed the PRT test on the right eye of 17 normal subjects on two consecutive days, and also found no significant difference between days. The 68% and 95% confidence limits for between-day repeatability were 5 mm/15 s and 10 mm/15 s respectively.

Cho and Kwong (1996), in a pilot study, compared the performance of the SP-CTT and the PRT test. They performed the two tests on 15 normal subjects on six different days over a period of two weeks. For each subject, one eye was measured using the PRT test and the other eye was measured using the SP-CTT. The priority of which eye received which test, and which eye being tested first was randomized. No significant between-day difference was found for either test.

2.1.5 Validity

Reports of the validity of CTT in the diagnosis of lacrimal deficiency lack coherence.

Kurihashi (1986) performed the CTTw (Method 1 - see Section 2.2.1 (i)) on both eyes of patients who had unilateral lacrimal impairment. In eight patients with "unilateral extratemporal facial nerve paralysis having lagophthalmos or weakness of orbicularis oculi muscle", a high ratio of wetting of the diseased side (with impaired lacrimal drainage function but normal tear secretion) to the normal side was consistently found. In seven patients with "unilateral acoustic tumors and one
patient with cholesteatoma in the left petrous apex", wet lengths obtained in the
diseased eyes were consistently smaller than the normal eyes.

Golding and Brennan (1993), however, found that the PRT test has very low
sensitivity (67%) and specificity (76%) in the diagnosis of keratoconjunctivitis
sicca (KCS). They also reported good correlation between the ST and the PRT
values. Owing to the advantages of the PRT test over the ST (see Section 2.1.2),
they suggested that the ST should be replaced by the PRT test.

Kwong and Cho (1998) compared the SP-CTT and the PRT test values between
46 dry eye and 37 normal HK-Chinese subjects. They found no significant
difference in the PRT values between the dry eye and the normal subjects, but the
SP-CTT values were significantly lower in the dry eye subjects. The SP-CTT
yielded sensitivity and specificity of 56.8% and 65.2% respectively when a cut-off
value of ≤6.5 mm/15 s was used, and 87.4% and 45.7% respectively when the cut-
off value was reduced to ≤4 mm/15 s. However, in their study, dry eye subjects
were recruited according to the diagnosis of ophthalmologists and they suggested
that not screening these subjects using specific criteria may have affected their
results.

Reports of the validity of the CTT in the identification of problematic contact lens
wearers have not been conclusive.

Hamano et al. (1983) measured the PRT value of 3336 eyes of contact lens
wearers, and reported that patients wearing soft lenses and with PRT value <9
mm/15 s had a higher chance of having dry eye symptoms. When there was a large
difference in the PRT value between the two eyes of an individual, clinical
examination often showed the eye with shorter PRT value to have symptoms of dry eye.

Cho and Yap (1995) measured the baseline SP-CTT value on 35 asymptomatic HK-Chinese non-contact lens wearers. All subjects were then fitted with a pair of soft lenses. After 28 weeks, they classified the subjects into three groups according to their complaints:

1. Group 1 - subjects with no complaints,
2. Group 2 - subjects who experienced sensation of dryness only after long periods (>10 hr) of contact lens wear,
3. Group 3 - subjects who complained of a definite sensation of dryness, with or without contact lenses, after commencing contact lens wear.

Subjects in Group 3 had very low baseline SP-CTT values, which were lower than those of Group 1 and 2. However, as they only had two subjects in Group 3, they tentatively concluded that the SP-CTT appeared to be able to predict the success of potential contact lens wearers. They suggested that the results needed to be confirmed with a larger group of subjects.

2.1.6 Statistical considerations

The distributions of various CTT values have been shown to be not significantly different from Normal (Kurihashi 1986, Sakamoto et al. 1993, Cho 1993a). No significant difference between the right and left eyes has been reported in any study, while significant correlation was found between the right and left eyes (Sakamoto et al. 1993). Herzberg (1983) commented, “The requirement that the observations in one-group and independent-groups studies be independent of each
other is absolute. Violation of this requirement in such studies definitely makes the analysis invalid". Statistical analysis using both eyes as individual data points, as did by Kurihashi (1986), Hamano et al. (1983) and Sakamoto et al. (1993), violates the assumption of independence and the validity of their results may be questionable on statistical grounds.

2.1.7 Comments

The CTT can be performed quickly and easily (Hamano et al. 1983), and is much less invasive than the ST. The SP-CTT and PRT test have been found to be reliable (Cho 1993a, Cho and Kwong 1996) and potentially able to screen out problematic contact lens wearers (Cho and Yap 1995).

However, performance of these tests is far from conclusive and the data are limited. Further investigations on the effect of contact lens wear, and on wetting value defined by the CTT and NITBUT (see Chapter 6) have been carried out and reported in detail in this thesis.
2.2 Non-invasive tear break-up time (NITBUT)

The TBUT test (see Section 1.2.2 (i)) is the most commonly used test for tear stability (Lemp et al. 1971, Lemp and Hamill 1973, Rengstorff 1974, Holly 1981a, Chopra et al. 1985, Guillon and Guillon 1988, Cho and Brown 1993). Some investigators have raised concerns that the instillation of fluorescein in this test may change the tear film stability, which may potentially affect the validity of the test (Mengher et al. 1985b, Norn 1986, Holly 1987, Tonge et al. 1991). Over the past 10 years, a number of non-invasive tests have been introduced. These tests depend on either the examination of the interference patterns on the corneal surface (tear film interferometry) (Guillon 1986), or the observation of the distortions of the reflected image of a grid pattern (Mengher et al. 1985a, Mengher et al. 1986a, Cho 1993b) or keratometer mire from the corneal surfaces (Patel et al. 1985, Hirji et al. 1989, Tonge et al. 1991). The non-invasive technique offers the advantages over the traditional TBUT test in that:

1. no fluorescein instillation is required,
2. high intensity illumination is avoided during observation (Bennett and Gordon 1989).

In this thesis, only tests involving the observation of the reflected image on the corneal surface will be discussed. Different acronyms have been used by different investigators, depending on the type of target or instrument used for assessing the non-invasive tear break-up time. To avoid confusion, in the following chapters, the acronym NITBUT will be used to refer to non-invasive tear break-up time measured using grid targets which project images covering over 50% of the cornea (but including the Tearscope method which uses a white uniform background (see
Section 2.2.1 (iv)).

2.2.1 Types of instrument used

2.2.1 (i) Xeroscope (NITBUT)

Mengher et al. (1985a) used a xeroscope mounted on a slit-lamp to measure the NITBUT. The xeroscope consisted of a polymeric hemisphere of 40 cm diameter with a grid pattern on the inner surface, and a shielded, fluorescent ring at the rim for illumination. To perform the test, the patient was asked to look at the center of the pattern and to refrain from blinking. The first Purkinje image of the grid lines was observed for disturbance from the slit-lamp via the objective lens at the apex of the hemisphere. Mengher et al. (1985a) suggested that, “A well-defined image of the grid implies the presence of a stable and intact tear film”. They also suggested that random distortions or discontinuities of the lines reflected from the cornea indicate destabilisation of the tear film: a distortion represents local thinning of the tear film (except those caused by the presence of debris or irregularities in the tear film), while a discontinuity represents a break up of the tear film. The time, in seconds, from the last blink to the first random appearance of discontinuity of the pattern was recorded as the NITBUT (they used the acronym NIBUT in their report). The image of the grid pattern covered nearly the whole visible cornea, but Mengher et al. only observed the central three quarters of the corneal surface for disruption, as they found that there was always distortion in the transition zone from the cornea to the limbus.
2.2.1 (ii) Keratometer (TTT)

Patel et al. (1985) performed similar procedures to those described above using a Bausch & Lomb keratometer. The major difference was that the target used in the test was the lower right mire of the keratometer, and the mire images only covered the central 3 mm of the corneal surface. They timed the interval between a blink and the appearance of a diffuse image of the mire and termed it the tear thinning time (TTT).

2.2.1 (iii) HIR-CAL grid (TP-RPT)

Hirji et al. (1989) also used the Bausch & Lomb keratometer to measure the tear stability. However, they replaced the mire with a specially designed grid, which they termed the Hirji-Callender (HIR-CAL) grid. A sheet of graph paper was magnified 2X and photocopied on to a black and white negative photographic paper. This was then cut to size and replaced the existing mire of the keratometer. The increased complexity of this pattern compared to the original mire allowed a more detailed assessment of disturbance within the central 3 mm of the cornea covered by the reflection of the grid. They suggested that tear film instability has two components: a tear film pre-rupture phase time (TP-RPT), as measured by the defocusing of the reflected mire image (termed TTT by Patel et al. (1985)) and the tear film rupture time.

2.2.1 (iv) Tearscope (NITBUT)

The Tearscope is an instrument specially designed to observe the tear film by Guillon (1986). It consists of a hemispheric cup with a small hole at the center
which allows observation through the eye piece (monocular) of a slit-lamp. The inner cup surface is illuminated by a cold cathode ring light source, so that the light source of the slit-lamp is not required during observation. One of its functions is to assess the NITBUT. In contrast to the methods using other instruments like the xerroscope, keratometer, HIR-CAL grid or the NITA instrument (see Section 2.2.1 (v)) which use black backgrounds, the Tearscope uses a white background to observe the appearance of black spot(s) within the specular reflection from the pre-corneal tear film (Guillon and Guillon 1989, 1994). The time from the last blink to the first appearance of a black spot is recorded as the NITBUT. Guillon and Guillon (1994) suggested that the white background method has the further advantage of allowing the observation of the tear film structure. The design of the Tearscope has been updated and is now commercially available (Keeler Tearscope-Plus) as a multi-use instrument for the non-invasive examination of the tear film (Guillon 1998). Measurement of the NITBUT can be performed with or without a grid pattern. Guillon (1998) suggested that the grid can be added when the lipid layer has a poor reflectivity, or in the presence of a light coloured iris where the observations are more difficult with the white field. The end point will then be the first appearance of a distortion or discontinuity of the grid pattern instead of a black spot. It was suggested that dark-field instruments do not measure the break-up time of the tear film at all, but, rather they measure the time when the tear film starts to destabililize or thin down (Patel et al. 1985, Hirji et al. 1989, Guillon and Guillon 1994). However, many investigators have measured NITBUT using the criterion of discontinuities observed on reflected grid pattern images on black backgrounds (see Section
2.2.2).

Young and Efron (1991) made an apparatus based on the design of the Tarscope, in which a curved white panel of 45 X 35 cm was used instead of the hemispheric cup of the Tarscope. The panel was illuminated by two vertical fluorescent tubes, one mounted on each side. To measure NITBUT, a grid of vertical and horizontal black lines on a white background was placed in front of the concave side of the panel. The measurement technique and end-point criterion used by Mengher et al. (1985a) were adopted (see Section 2.2.2). The cornea of the subject was observed through a hole in the center of the panel, through the magnifying system of the slit-lamp. The illuminators were masked so that their reflection could not be seen in the cornea. This is another white background NITBUT instrument, and Guillon and Guillon (1994) called it the ‘External illuminator’.

2.2.1 (v) NITA instrument (NITBUT)

Cho and Brown (1993) designed a portable non-invasive tear assessment (NITA) instrument, which is similar to the xeroscope used by Mengher et al. (1985a) to measure the NITBUT. A black wok of 30 cm diameter was mounted on a wooden stand. White concentric rings, about 0.2 cm thick and 1 cm apart, were drawn on the concave surface of the wok. Radiating lines were also drawn from the center to the periphery to form a grid pattern. A hole of 1 cm diameter was drilled at the center of the wok, where a +10.00 DS lens and a telescope were placed on the convex side of the wok. A circular fluorescent lamp (Philips, TLE, 32W, tube diameter 28 mm, color 54 cool daylight) was attached to the periphery of the wok for illumination. The fluorescent lamp was shielded to prevent glare and heat. The
image was observed via the telescope, and the measuring procedure was similar to that used in Mengher et al.'s study (1985a), except that the end point was distortion or discontinuity of the reflected image on the cornea (see Section 2.2.2). The coverage of the visible cornea of the subject was 75-85%, depending on the horizontal visible iris diameter of each subject. Part of the image may also be blocked by the nose and lashes (Cho 1993b).

Measurements of the TTT and the TP-RPT using the keratometer and HIR-CAL grid have the main disadvantage that only a small central area (of diameter about 3 mm) of the cornea is covered, so any disturbance of the tear film in the peripheral cornea will be missed. One would expect longer values for TTT and TP-RPT but, on the contrary, longer values have been reported for NITIBUT using the xeroscope (see Table 2.2). This may be because the xeroscope shields the subject's eye, which reduces the ventilation to the eye, and hence reduces evaporation of tears, leading to increased tear stability. Another reason may be that different end-point criteria were used for these techniques (see Section 2.2.2).

2.2.2 End-point criteria

Mengher et al. (1985a) used discontinuity of the reflected grid image of the xeroscope as the end point. Using this end-point criterion, NITIBUT values as long as 3 to 5 min have been observed. An arbitrary cut-off time of 30 s was used on the grounds that extended exposure of the ocular surface is likely to stimulate tear flow and alter tear film stability (Mengher et al. 1985a).

Patel et al. (1985), in their study, defined the end point of their TTT measurement (see Section 2.2.1 (ii)) as diffusion of the reflected image of the keratometer mire
while keeping the image in focus continuously. But in most studies of Patel and co-workers (Patel et al. 1988, Patel and Farrell 1989, Patel et al. 1991a and 1991b), the end-point of diffusing or doubling (defocusing) of the mire image was used for their TTT measurements. Patel et al. (1985) did not set a cut-off time, and their reported results were mostly less than 30 s.

The end-point criteria used by Hirji et al. (1989) for their TP-RPT measurements (see Section 2.2.1 (iii)) were defocusing and distortion of the reflected image of the HIR-CAL grid. The values they obtained were in good agreement with the TTT values obtained by Patel et al. (1985), who used a similar end-point criterion. They suggested that both the TP-RPT and the TTT were measuring the same phase (pre-rupture phase) of the tear film. This may account for the good agreement found between the TP-RPT and the TTT measured by Hirji et al. (1989) and Patel et al. (1985) respectively, although the HIR-CAL grid method may be expected to be more sensitive due to the increased detail of the target within the central 3 mm. However, their results were shorter than the NITBUT values reported by Mengher et al. (1985a), who used discontinuity as the end-point criterion. Hirji and co-workers suggested that the reason why their measurements were shorter than the NITBUT measured by Mengher et al. (1985a) was that the TP-RPT and the TTT were measuring the pre-rupture phase of the tear film whereas the NITBUT was measuring the rupture phase.

Young and Efron (1991), using the appearance of a break forming in the corneal reflection of the grid as end point, found NITBUT of >20 s in both eyes of six healthy subjects. No cut-off time was adopted in their study.

Tonge et al. (1991) used "wide spread distortion" of the reflected grid image of
the xeroscope as the end-point. A cut-off value of 180 s was adopted. Tonge et al. (1991) obtained a median NITBUT of 62 s, which was substantially longer than the values reported by other investigators (see Table 2.2). This was probably due to the use of "widespread distortion" as their end-point. It is unclear how they defined 'widespread distortion'.

Faber et al. (1991) used both the distortion and the discontinuity criteria to measure the NITBUT of subjects before and after contact lens wear. They reported that the results obtained using the distortion criterion "paralleled" those obtained using the discontinuity criterion. They also observed that the reflected image may distort without leading to discontinuity, and discontinuity may occur without a prior distortion. They suggested that using the discontinuity criterion "provides a more definitive cut-off point" than using the distortion criterion since the latter is open to interpretation.

Cho (1993b) used distortion or discontinuity of the reflected grid image of the NITA instrument as the end point, but they excluded any distortions which resolved without leading to breaks of the image as these were considered to be due to movements of debris. No cut-off time was set.

Little and Bruce (1994), in a study of the repeatability of TTT using the Bausch & Lomb keratometer, used not only distortion or defocus, but also discontinuity of the reflected mire image as the end-point.
<table>
<thead>
<tr>
<th>Investigator (Year)</th>
<th><em>No. of measurements</em> (subjects)</th>
<th>Type of observation (Instrument)</th>
<th>Cut-off value (s)</th>
<th>End-point criteria</th>
<th>Median (s)</th>
<th>Mode (s)</th>
<th>Mean(SD) (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patil <em>et al.</em> (1985)</td>
<td>60 (6)</td>
<td>TTT (Keratometer)</td>
<td>-</td>
<td>Defocusing</td>
<td>16.0</td>
<td>-</td>
<td>18.0(6.5)</td>
</tr>
<tr>
<td>Mengher <em>et al.</em> (1985a)</td>
<td>88 (9)</td>
<td>NITBUT (Xeroscope)</td>
<td>30</td>
<td>Discontinuity</td>
<td>29</td>
<td>-</td>
<td>47.9</td>
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<tr>
<td>Hirji <em>et al.</em> (1989)</td>
<td>90 (9)</td>
<td>TP-RPT (HIR-CAL grid)</td>
<td>-</td>
<td>Defocusing</td>
<td>15</td>
<td>-</td>
<td>18.6(11.0)</td>
</tr>
<tr>
<td>Guillen &amp; Guillen (1988)</td>
<td>(121)</td>
<td>NITBUT (Tearscope)</td>
<td>45</td>
<td>Appearance of dark spot</td>
<td>-</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>Tonge <em>et al.</em> (1991)</td>
<td>81 (27)</td>
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<td>Wide spread distortion</td>
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<td>-</td>
</tr>
<tr>
<td>Cho (1993b)</td>
<td>(7) HK-Chinese</td>
<td>NITBUT (NITA)</td>
<td>-</td>
<td>Discontinuity or distortion (leading to a discontinuity)</td>
<td>15.5</td>
<td>17.5</td>
<td>16(9.4)</td>
</tr>
<tr>
<td>Brown &amp; Cho (1994)</td>
<td>(52) HK-Chinese</td>
<td>NITBUT (NITA)</td>
<td>-</td>
<td>same as Cho (1993b)</td>
<td>-</td>
<td>3.2</td>
<td>15.6(13.8)</td>
</tr>
<tr>
<td>Little &amp; Bruce (1994)</td>
<td>(17)</td>
<td>TTT (Keratometer)</td>
<td>60</td>
<td>Distortion, discontinuity or area of defocus</td>
<td>24</td>
<td>-</td>
<td>26.5(15)</td>
</tr>
<tr>
<td>Madden <em>et al.</em> (1994)</td>
<td>(45)</td>
<td>NITBUT/ TP-RPT (Xeroscope/ HIR-CAL)</td>
<td>60</td>
<td>Discontinuity (including those caused by tear film debris) or deterioration of target mire</td>
<td>-</td>
<td>-</td>
<td>Approximate mean: Xeroscope: 44.7(16.3) Keratometer: 35.6(19.2)</td>
</tr>
<tr>
<td>Cho &amp; Yap (1995)</td>
<td>(35) HK-Chinese</td>
<td>NITBUT (NITA)</td>
<td>-</td>
<td>Same as Cho (1993b)</td>
<td>5.6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Presumably Caucasian subject unless specified.
Madden et al. (1994) used discontinuity of the HIR-CAL grid image, defined as not only breaking of grid lines but also lines which "was not ameliorated" by refocusing the instrument, as the end point. However, it is unclear whether "not ameliorated" included blurring (defocusing) of lines. Their end point also included discontinuity of the reflected image caused by debris in the tear film. In their study, the xeroscope and the keratometer method were compared using the same criteria. A cut-off time of 60 s was adopted. The mean value they obtained by the keratometer method was substantially longer than the reported values for TTT and TP-RPT by previous investigators (see Table 2.2.1). They commented that, "the HIR-CAL technique gave clinically (as opposed to statistically) comparable result to the xeroscope method".

2.2.3 Reliability

There is no consensus on the reliability of the tests used for assessing non-invasive tear film stability. It is difficult to draw a conclusion as different investigators had used different ways of determining reliability. The statistical tests used by some investigators were questionable as data from the two eyes of each subject had been treated as independent data.

Little and Bruce (1994) used the Bausch & Lomb keratometer to measure the TTT of 17 normal young Caucasian subjects on two consecutive days. Each time, three readings were taken and the mean value was used as the representative TTT. They tested the reliability of the TTT by analyzing the distribution of the individual difference of TTT values between the two days, and calculating the ratios for 95% confidence intervals of the TTT values. Since they observed an increase in the
between-day differences with the individual means, the data were transformed using natural logarithms. They found that the ratios for the 95% confidence intervals of the TTT were 3.8 and 0.2. Thus, for a subject with a TTT of 15 s, there was a 95% probability that a subsequent result would lie between 57 and 3 s. They reported that the TTT test was not sufficiently reliable for clinical use.

Cho (1993b) tested the reliability of the portable NITA instrument on seven normal asymptomatic HK-Chinese. Measurements were made on eight separate days over a period of eight weeks and five NITBUT readings were taken for each subject at each visit. Using Friedman two-way analysis of variance, Cho reported substantial variation and unreliable results when gross data were used. However, when the average of the three closest readings out of five readings for each subject was used as the representative NITBUT, the variation was markedly reduced and reliable results were obtained. In another study, Cho commented that, “High values may be due to delayed measurements and low values may occur because the tear film has not spread smoothly after the blink just before measurement”.

The delayed measurements were explained to be due to the reduced coverage of cornea by the visible reflected image (see Section 2.2.1 (v)), and any tear disruptions in these “shadowed” areas will be missed. Cho suggested that this justified the omission of extreme values (Cho 1993b).

Madden et al. (1994), on the other hand, found that the Bausch & Lomb keratometer could produce reliable TP-RPT readings (see Section 2.2.1 (iii)). Thirty-two normal subjects were assessed to investigate the repeatability of the NITBUT (xeroscope) and TP-RPT. For each subject, three TP-RPT and NITBUT readings were taken at each of two visits, and the data obtained on the two days
were compared using the generalized sign rank test. They found that both techniques showed excellent repeatability. To investigate inter-observer variation, two measurements were taken with both techniques by two observers on another eight subjects on the same afternoon. In both techniques, significant variations were found between the results obtained by the two investigators, indicating a potential inter-examiner error in the study of NITBUT when more than one examiner performed the test. They concluded that the same-subject, same-investigator repeatability with both techniques were excellent. The increased TP-RPT reliability of the keratometer method was probably due to the increased details of the target pattern (HIR-CAL grid - see Section 2.2.1 (iii)), compared with the empty rings of the Bausch & Lomb keratometer mire. However, they treated the data from the same eye of each subject as independent, which may compromise the validity of their analysis (see Section 2.2.7).

2.2.4 Validity

Based on the limited reports available, dry eye subjects were found to have shorter NITBUT than normal subjects. Again, it is difficult to draw a conclusion as only reports by a few investigators were found, and they combined values obtained from both eyes of each subject for analysis.

Mengher et al. (1985a) obtained NITBUT values from 12 dry eye subjects and nine normal subjects. Two measurements were obtained from each eye alternating between eyes, with the right eye always assessed first. The procedures were repeated on five consecutive days for the normal subjects while they were performed in a single visit only for the dry eye subjects. Out of a total of 176
observations, combining data from the right and left eyes, 67% of the NITBUT readings were longer than 20 s. In contrast, none of the dry eye subjects had NITBUT longer than 20 s. They commented that, "the non-invasive instrument provides an alternative method of assessing tear film stability and it should be valuable in the study of artificial tear substitutes in dry-eyes".

Mengher et al. (1986a) investigated the sensitivity and specificity of the NITBUT test in the diagnosis of dry eye in Caucasian subjects. They obtained NITBUT of 33 dry eye subjects (65 eyes) and 66 normal subjects (132 eyes), twice for each eye and alternating between eyes, with the right eye always assessed first. Using a critical value of 10 s, the test provided a sensitivity of 82% and a specificity of 86%. They defined 'dry eye subjects' in their study according to the following criteria:

1. Symptoms of ocular surface irritation compatible with the dry eye state.
2. Interpalpebral punctate staining with fluorescein of the conjunctiva and cornea when viewed with a blue excitor and yellow barrier filter.
3. ST value (without topical anaesthetic) of less than 10 mm of wetting on at least two occasions.

It is unclear if, and how they graded the level of interpalpebral punctate staining on the corneal surface. The use of ST is also questionable as it has been widely criticized as unreliable (see Section 2.1). They also treated data from both the right and left eyes of the same subject as independent in their analysis. Hence, the validity of their result may be questionable on statistical grounds (see Section 2.2.7).
2.2.5 Factors affecting NITBUT

Since the NITBUT test is a comparatively new technique (compared to the TBUT test), there are relatively fewer reports of the effects of different factors on NITBUT. The following presents a summary of some studies reporting the effects of different factors on NITBUT.

2.2.5 (i) Age and Gender

Non-invasive tear stability has been reported to decrease with increasing age, and no significant difference was found between genders.

Patel and Farrell (1989) measured the TTT values (see Section 2.2.1 (ii)) of 123 normal subjects (42 males and 81 females) aged from 8 to 80 years. A significant linear reduction in TTT was found within the age range. They found no significant difference in TTT between males and females.

Tonge et al. (1991) measured the NITBUT of 27 subjects (13 males and 14 females) aged from 13 to 67 years. Fifteen of the subjects were aged less than 30 years and 12 of them were aged more than 30 years. They found a significantly lower NITBUT value in the older (median NITBUT=38 s) than in the younger group (median NITBUT=70 s). No significant difference was found between males and females. However, it should be noted that Tonge et al. used data from both eyes (three measures from each eye) of the same subject in their analysis. The validity of their result may therefore be questionable (see Section 2.2.7).

2.2.5 (ii) Diurnal variation

Patel et al. (1988) reported a low TTT upon awakening followed by a steady level
subsequently. They measured the TTT of the right eyes of 10 subjects from 8:00 am to 8:00 pm at 2-hour intervals. Each measure consisted of 10 values which were then averaged. The TTT was found to be lowest at 8:00 am and did not vary significantly between 10:00 am and 8:00 pm. However, the pattern of change differed from subject to subject.

They suggested that the low TTT value at the early morning may be due to one or more of the following factors:

(1) a low mucin level,

(2) a low lipid level,

(3) an altered corneal epithelial surface.

However, in an earlier study, Kessing (1966) reported that the tear mucin level in a sleeping subject was twice as high and would decrease rapidly upon awakening.

To our knowledge, there is no study reporting the diurnal variations in the human tear lipid level.

Patel et al. (1988) used two-way analysis of variance (ANOVA), which is a parametric test, for analyses of their data although the distribution of TTT has been reported to be non-Gaussian (Patel and Farrell 1989) and hence, non-parametric tests would be more appropriate (see Section 2.2.7), especially since their sample size was small (10 subjects). Parametric tests, which use the means as reference points for comparisons may be subject to the effects of extreme values and yield inaccurate result (see Section 2.2.7).

2.2.5 (iii) Right versus left eye.

Investigators agreed that there is no significant difference in the tear stability
between the right and the left eyes.

Mengher et al. (1985a) measured the NITBUT of nine normal subjects. Each eye was measured twice in alternating sequence between the right and the left eye, with the right eye always measured first. Each subject was studied on five consecutive days and each observation was treated as an individual data point (see Section 2.2.7). The mean NITBUT values were 47.9 s and 35.1 s for the right and left eyes respectively, and the difference was not significantly different.

Hirji et al. (1989) obtained 90 TP-RPT (see Section 2.2.1 (iii)) from the right eyes and 85 measurement from the left eyes of nine subjects. The right eye was always measured first. They used t-tests to compare the results and found no significant difference. However, the use of a parametric test and treatment of the repeated measurements on the same subjects as independent data was questionable on statistical grounds.

Tiffany et al. (1989) also reported no significant difference between the right and the left eyes of 37 asymptomatic subjects. But they did not provide the results of their statistical analysis.

Tonge et al. (1991) measured the NITBUT of both eyes of 27 subjects. Three measurements were made on each eye. The right eye was always measured first and then alternated between the right and the left eyes. No significant difference was found between the right and the left eyes. However, it is unclear why they used Mann-Whitney 'U' test instead of Wilcoxon signed-rank test (see Section 2.2.7).
2.2.5 (iv) Blink rate

It has been reported that blink rates are reduced when performing visual tasks such as using a VDU, or during reading, while the NITBUT is not affected by such visual tasks. However, contradictory results have been reported on the relationship between blink rates and the non-invasive tear stability.

Patel et al. (1991a) investigated the effect of changes of the blink rate on TTT associated with the use of VDU in 16 subjects. Blink rates of each subject were measured during a conversation, and then during the use of a VDU when the subject was playing a computer game. One investigator was responsible for counting the blinks by direct observation, while the other measured the time. TTT values were obtained before and immediately after using the VDU. The assumption was that TTT during VDU use was indirectly reflected by the TTT measured immediately after using the VDU. Patel et al. reported,

(a) no significant correlation between inter-blink interval and TTT before VDU use (Spearman r=0.196),

(b) a significant correlation between inter-blink interval and TTT during VDU use (Spearman r=-0.621).

Using the raw data provided in the report of Patel et al., Cho (1995) reported that there appeared to be an error in their report; there was a significant correlation between blink interval and TTT, as reported by Patel et al., but the Spearman correlation coefficient for (b) should be positive. The probability value for (a) was 0.469, and for (b) was 0.01; there was a highly significant difference between blink rates before and during VDU use (t-test for related samples; t=-11.8, df=15, p=0.00).
Cho et al. (1997) investigated the effect of reading Chinese and English articles on NITBUT and the inter-blink interval. Thirty-three HK-Chinese subjects were recruited. Inter-blink intervals were calculated indirectly from the blink rates, which were measured by an examiner without the subjects' awareness. The NITBUT values were measured before reading and immediately after reading a Chinese article and an English article respectively. Five NITBUT measurements were taken each time, and the average of the closest of three readings was used as the representative NITBUT. They found no effect of reading on the NITBUT but a significant decrease in the blink rates. They also found a significant correlation between NITBUT and inter-blink interval before reading and when reading the Chinese article, but no correlation when reading the English article. Hence, no firm conclusion could be drawn regarding the relationship between blink rates and NITBUT during or after performing a visual task.

2.2.5 (v) Iris colour

Patel et al. (1991b) compared the TTT between 10 blue-iris subjects and 10 brown-iris subjects, and found that the TTT of the latter group were significantly longer than those of the former group. However, they used a parametric test for analysis (see Section 2.2.7). Although their data were normally distributed, it may not be appropriate to use parametric test as their sample size was fairly small (n=10). When the data provided by Patel et al. were reanalysed using Friedman two-way ANOVA, no significant difference in TTT between the two groups was found (Cho 1995).
2.2.5 (vi) Instillation of fluorescein

It has been suggested that instillation of fluorescein reduces the tear stability which is the main concern when using the TBUT test. However, studies of the effect of fluorescein instillation on tear stability measured by non-invasive techniques have reported contradictory results.

Patel et al. (1985) measured the TTT on the right eyes of six subjects (10 measurements each) before and after instillation of fluorescein. The mean TTT values were 18 s and 14.4 s respectively, and the difference was statistically significant. They concluded that the instillation of fluorescein destabilised tears. However, they used parametric tests for their analysis, and they treated each measurement as separate data (see Section 2.2.7), and the experiment was not performed with the examiner masked.

Mengher et al. (1985b) measured the NITBUT on nine subjects before fluorescein instillation (three times on each eye), and then 0-2 min, 3-5 min, and 10-20 min after fluorescein instillation (twice on each eye at each testing time). Only one eye had fluorescein instilled, while the other eye was used as a control. A yellow filter was used in the observation path so that the examiner was masked as to which eye was treated with fluorescein. They reported that over 80% of the NITBUT values before the instillation of fluorescein were longer than the 30 s cut-off value used in the study. The proportion was reduced to about 35% immediately after fluorescein instillation, and then increased again as the measurement time after instillation of fluorescein was increased. They reported that NITBUT returned to baseline 10 to 20 min after fluorescein instillation. In their study, they appeared to treat the readings of each individual measurement as separate data, and Mann-
Whitney 'U' tests were used in their analysis (see Section 2.2.7).

In a single-masked study, Cho et al. (1996b) investigated the effect of fluorescein on NITBUT on 24 asymptomatic HK-Chinese. For each eye, they took five readings. The average of the three closest readings out of each five readings was used as the representative pre- and post-fluorescein instillation NITBUT of the eye. An arbitrary cut-off time of 60 s was used, and the post-fluorescein instillation NITBUT was measured within 40 s after the treatment with fluorescein. They found no significant difference in the NITBUT between the pre- and post-fluorescein instillation. The Wilcoxon signed-rank test was used for their analysis.

2.2.5 (vii) Instillation of local anaesthetic and saline

For subjects who cannot refrain from blinking before the end point during NITBUT measurement, it is possible to use local anaesthetic to help them keep their eyes open. However, it has been suggested that the instillation of local anaesthetic may cause corneal epithelial damage and thus reduce the stability of the tear film. Some investigators have suggested that the effect is probably due to the preservative (such as benzalkonium chloride) rather than the anaesthetic itself (Wilson et al. 1975). Previous studies have supported the idea that the tear stability will not be affected by the use of unpreserved anaesthetic, if there is a delay of 5 min or more after instillation.

In a double masked experiment, Mengher et al. (1986b) assessed the NITBUT of 12 normal subjects 5 min before, and then 1, 3, 5, 10 and 12 min after instillation of saline to the control eye and 0.4% oxybuprocaaine hydrochloride to the test eye.
No significant difference was found in the NITBUT between the two eyes. NITBUT values measured at different times were not significantly different, and the increased volume of fluid in the tear film did not affect the NITBUT value. Mann-Whitney 'U' tests were used for all their analyses (see Section 2.2.7).

In a masked experiment, Patel et al. (1991b) measured the TTT of the right eyes of 20 young adults before and after topical anaesthesia using two kinds of unpreserved local anaesthetic (0.4% benoxinate hydrochloride and 0.5% amethocaine hydrochloride). Ten of their subjects had blue irides and the other 10 had brown irides. The TTT measurements were performed either before and 5 min after instillation of the anaesthetic, or 5 min after the instillation of the anaesthetic, and then about 20 min later, after the anaesthetic had “worn off”. With anaesthetic, a significant reduction of TTT was found in blue-eye subjects only. Parametric tests were used in their analyses although their sample size was small (see Section 2.2.7). When their data were reanalysed using non-parametric tests, no significant difference in the TTT between subjects with different iris colour, with or without anaesthetic was found (Cho 1995) (see Section 2.2.5 (v)).

Cho and Brown (1995) measured the NITBUT in 22 asymptomatic young Chinese adults before and after instillation of unpreserved benoxinate (0.4%) and saline. The experiment consisted of three parts. In the first part, NITBUT values were measured 30 s after instillation of anaesthetic on one eye and no treatment on the other eye; in the second part, NITBUT measurements were performed 5 min after instillation of anaesthetic on one eye and saline on the other eye; and in the third part, the measurements were performed 30 s after instillation of saline on one eye and no treatment on the other eye. All experiments were conducted with the
observer masked with respect to the eye which had received the anaesthetic. They found that NITBUT was significantly increased 30 s after instillation of anaesthetic, and this increase was no longer found if the NITBUT was taken 5 min after the instillation. Increased fluid volume by the instillation of saline had no significant effect on NITBUT. Wilcoxon signed-rank tests were used in their analyses.

Blades et al. (1999) commented that previous studies “have been limited by the inability to measure tear stability and the pharmacological activity of the drug in parallel”. In a masked experiment, they used a Non-Contact Corneal Aesthesiometer, a non-invasive device which uses a jet of air as the stimulus, to assess corneal sensitivity in parallel with the measurements of the TTT before and after instillation of unpreserved 0.4% benoxinate hydrochloride. TTT and corneal sensitivity were measured on 20 subjects at different time intervals after instillation: 5, 10, 15, 20, 30, 45 and 60 min. The drop was instilled in the right eye, left eye or both eyes on three different days on all subjects. The tests were also performed on 10 subjects without any treatment to serve as control and to obtain baseline values. They found that the anaesthetic used did not significantly affect the TTT. They concluded that unpreserved 0.4% benoxinate hydrochloride could be used to facilitate tear film stability assessment. However, they suggested that the results may be influenced by the use of different tear stability assessment techniques or the composition of the anaesthetic used. Whether the anaesthetic is preserved or not may also be an important factor.
2.2.5 (viii) Hydrogel contact lens wear

A decrease in NITBUT followed by a recovery has been observed after hydrogel contact lens wear.

In a double masked study, Faber et al. (1991) investigated the effect of hydrogel lens wear on the NITBUT of six subjects. Each single NITBUT measurement consisted of two readings according to the two end-point criteria they used, distortion and discontinuity (see Section 2.2.2). Six normal subjects were recruited and measurements were made on both eyes of each subject. Baseline NITBUT was measured on one eye before contact lens wear, then a contact lens was placed on the eye. The same procedure was repeated on the other eye after 20 min. The contact lenses used were Bausch & Lomb Optima lens which has a water content of 38%, and Igel 67 lens which has a water content of 67%. One of these two lens types was used on each eye randomly. After one hour of lens wear, the lens was removed and NITBUT was measured at 1, 5, 15 and 60 min. They found that the baseline NITBUT was significantly longer than the NITBUT at 1 and 5 min after lens removal, but not thereafter. Parallel results were obtained with the two end-point criteria, and the type of lens used did not affect the post-contact lens wear NITBUT. The time required for 95% recovery, after lens removal, was about 26 min. However, as they used parametric tests, even though their sample size was very small, their results may be questionable (see Section 2.2.7).

Cho and Yap (1995) (see Section 2.1.5) measured the NITBUT of 35 HK-Chinese before and after 2, 9 and 28 weeks of soft contact lens wear. They found that NITBUT values decreased after 2 and 9 weeks but not after 28 weeks of contact lens wear.
2.2.6 NITBUT and contact lens wear

Reports of NITBUT for predicting success in contact lens wear are scarce. Tonge et al. (1991) suggested that prospective lens wearers who exhibited a pre-fitting NITBUT of less than 20 s should be carefully monitored, but it is unclear how they reached this conclusion.

In a longitudinal study, Cho and Yap (1995) (see Section 2.1.5) compared the baseline NITBUT of 35 HK-Chinese who were finally classified into three groups, according to the number of hours they could wear their soft contact lenses without signs and symptoms. They found that baseline NITBUT value was not useful for identifying subjects who were likely to be successful soft contact lens wearers.

2.2.7 Statistical considerations

Measurements from the two eyes of a subject are usually significantly correlated (see Section 2.2.5 (iii)) and thus cannot be treated as independent. Measurements of the same subject at different time intervals are also not independent since they will show less variability than the same number of data values obtained from different subjects. Newcombe and Duff (1987) stated that, “As a general rule, any significance test in which the implied total ‘sample size’ exceeds the number of subjects in the study is invalid”.

Mengher et al. (1985a, 1986a) measured the NITBUT twice on each eye of each subject and treated each observation as separate data value. They also used Mann Whitney ‘U’ tests instead of Wilcoxon signed-rank tests for comparison of results within subjects. Tonge et al. (1991) measured the NITBUT three times on each eye of each subject. They also treated all the data as independent.
The results of the majority of studies on NITBUT showed that the NITBUT values are not normally distributed (Mengher et al. 1985a, Hirji et al. 1989, Patal et al. 1989, Tonge et al. 1991, Cho and Brown 1993). Parametric tests assume that data are normally distributed, so when used with data which have non-Gaussian distributions, inaccurate results, leading to possibly inappropriate conclusion may result. Non-parametric tests, on the other hand, do not make very serious assumptions on the distributions of the data. The median rather than the mean is used as a reference point for comparison so that the result is unlikely to be affected by extreme values. The sample size can also have a large impact on the validity of the statistical analysis when parametric tests are used since the mean is used as a reference point for comparison. With small samples, the data will be more subject to the effects of extreme values. In some studies (Hirji et al. 1989, Faber et al. 1991, Patel et al. 1991b), although the non-invasive tear stability data were normally distributed, the sample sizes were small (≤10) but the investigators used parametric tests for their analyses. Validity of their analyses were thus questionable as any extreme values from one or more subjects may result in a misinterpreted outcome.

2.2.8 Comment

Since there were different opinions on the mechanism of the tear film rupture (Mishima and Maurice 1961, Holly 1973, Lin and Brenner 1982, Sharma and Ruckenstein 1985), and what the different non-invasive tear stability techniques are really measuring are still under debate (see Section 2.2.1(iv)), we are still unable to get to grips with what these techniques do and how they work.
Nevertheless, the changes of the reflected images as observed at the end points of these techniques are obviously referring to changes in regularity of the tear film (Mengher 1985a) which may somehow reflect the tear film stability (see Section 2.2.1(i)).

The main advantage of the NITBUT test is that no fluorescein instillation is required, so that the tear film will not be disturbed during the test. However, there is no evidence which supports its usefulness in screening out patients who will be likely to suffer from dry eye problems if they start wearing contact lenses. Although some investigators have reported this test to be useful for the screening of dry eye patients, the evidence is not conclusive. To date, there is no report of a study combining this test with other tests of tear volume, such as the CTT, to predict contact lens wear outcomes. Before firm conclusions can be made about the usefulness of this test, more investigations have to be carried out with more carefully designed methodologies and appropriate sample sizes.
2.3 Wetting value

Fanti and Holly (1980) defined a wetting value by an equation, which combined the TBUT and ST value:

\[ \text{Wetting value} = f1 \text{ (TBUT)} + f2 \text{ (ST)}, \]

where \( f1 \) and \( f2 \) are both numerically equal to 1, to render the value dimensionless. They suggested that either a low TBUT value or ST value alone may not necessarily cause contact lens problem provided that the other factor was large enough to compensate. The higher the wetting value, the better the contact lens wearing performance. However, since the reliability of the ST is questionable (Pinschmidt 1970, Feldman and Wood 1979, Cho and Yap 1993), the validity of this equation is also doubtful (Loran et al. 1987). Although the CTT has been introduced for a number of years, there does not appear to be any study reporting an assessment of tear function using CTT value in combination with other tear tests. In view of the comparatively higher reliability of the CTT (Cho 1993a) and the advantages of the CTT (see Section 2.1.2) and the NITBUT (see Section 2.2.) over the ST and the TBUT test respectively, a study investigating the combinations of these values needs to be done.
Chapter 3

INTRODUCTION TO EXPERIMENTAL STUDY AND RESEARCH AIMS

3.1 The tear film and contact lens wear

There is a dual relationship between the precorneal tear film and contact lens wear. Inadequate quantity and poor quality of the tear film can interfere with successful contact lens wear, and the contact lens in vivo may somehow alter the integrity of the tear film. It has been reported that the most common complaint of contact lens wear is ‘dryness’ (Brennan and Efron 1989). This results in reduced wearing time or even drop-outs from lens wear. It has been suggested that this symptom is related to a deficient tear film and to dehydration of the lenses during lens wear. It is important to evaluate the precorneal tear film before contact lens fitting so as to screen potential problematic contact lens wearers and provide appropriate advice and care.

The criteria of a number of clinical tests for the diagnosis of dry eye are well documented. However, practitioners have noted that many contact lens wearers complaining of dryness after commencing lens wear may be free of any signs and symptoms before the commencement of contact lens wear. It seems that traditional tear tests such as the ST and TBUT are not sensitive enough to screen out such ‘marginal’ dry eye patients. Over recent years, modified techniques of tear tests, namely, the NITBUT test and the CTT (PRT and SP-CTT - see Section 2.1.1) allow non-invasive and less invasive assessments of the tear film stability and tear volume respectively. They have claimed to be more valid than the traditional versions of these tests and were thus believed to be more sensitive to detect a milder form of dry eye.
3.2 Contact lens wear in the Chinese population

The number of contact lens wearers is on the increase in Asia, especially in China. The lens type being fitted is predominantly the soft (hydrogel) lens. In Hong Kong, it has been reported that over 86% of the contact lens wearers use soft lenses (Cho and Conway 1994). However, little has been known about the characteristic of the tear film and its relationship with contact lens wear in this population until recent years.

Since 1993, Cho and co-workers have performed a series of studies on the tear film stability and tear volume in the Chinese eyes. Their results suggested that the NITBUT test and the SP-CTT are reliable in the assessment of tear film stability and tear volume respectively in Chinese eyes. Cho and Yap (1995) (see Section 2.2.5 (viii) and 2.1.3 (ix)) found that soft contact lens wear could cause a transient reduction of NITBUT, but had no effect on SP-CTT value. When used in isolation, the SP-CTT appeared to be more useful in predicting contact lens wearers who may have symptoms after wearing soft contact lenses while the NITBUT test was not (see Section 2.1.5 and 2.2.6). However, due to the small number of subjects in their study, it is necessary to confirm their findings with a larger sample size.

3.3 Aims of the current study

It is time to confirm the usefulness of the NITBUT, SP-CTT and PRT tests in predicting success of contact lens wear, and to determine the optimal cut-off value which can be used as the screening criterion for potential contact lens wearers. It would also be of interest to know whether these tests can give a better prediction of success in contact lens wear when used in combination. Besides that, the
potential impact of seasonal change of humidity on the test results should also be investigated. The current study was therefore designed with the following aims:

a) to investigate the effect of contact lens wear on the tear stability (NITBUT) and tear volume (SP-CTT and PRT values) after lens removal in a larger group of HK-Chinese,

b) to determine whether the tests, in isolation or in combination, can be used to predict successful contact lens wear, and if so,

c) to determine the optimal cut-off criterion and the corresponding accuracy of these tests, in isolation or in combination, in predicting successful contact lens wear,

d) to investigate seasonal change on the tests values, and dry eye-related problems in contact lens wear.
Chapter 4

INVESTIGATION TECHNIQUES

4.1 Tests used

(i) Non-invasive tear break-up time (NITBUT) test.

(ii) Self prepared-cotton thread test (SP-CTT).

(iii) Phenol red thread (PRT) test.

(iv) Subjective assessment of dry eye-related symptoms during contact lens wear using standard set of questions.

(v) Ocular examination using slit-lamp biomicroscope and grading using Efron’s scale.

Figure 4.1 The NITA instrument (front view).
4.2 Material and procedure of each test

4.2.1 NITBUT test

Material: NITA (non-invasive tear assessment) instrument (Cho 1993b)

A white grid pattern was drawn on the concave surface of a small black wok (a deep, round Chinese cooking pan, see Figures 4.1&4.2) of diameter 30 cm and radius of curvature 20.6 cm, using white paint marker pen. The grid lines were about 0.3 mm thick and the pattern was composed of white concentric rings 0.5 cm apart at the central 8 cm region and 1 cm on the outer region, and radiating lines from center to periphery. The spherical convex mirror nature of the central area of the human cornea caused a “barrel distortion” to the reflected image which increased the separation between the grid lines image of the original grid pattern. This was compensated by increasing the number of circular and radial lines to the central 8 cm portion of the grid pattern (see Figure 4.1). A circular fluorescent lamp (Philips, TLE, 32 W, tube diameter 28 cm, color 54 cool daylight) was
attached to the periphery of the wok. The surface of the fluorescent tube (facing the subject) was shielded with black, opaque paper to prevent glare and to minimize any heat from the fluorescent tube from affecting the evaporation of the tears.

![Eye with reflected grid image](image)

**Fig. 4.3 Reflected grid image of the NITA instrument on the cornea for assessing NITBUT.**

The grid pattern illuminated by the fluorescent tube was projected onto the subject’s eye and a clear, regular grid image was observed on the tear surface. The degree of image coverage on the cornea depended on the horizontal and vertical visible iris diameter of the subject, the position of the upper lid and size of the subject’s nose (see Figure 4.3).

The wok was mounted in a wooden frame which acted as a stand, and this set-up (wok and stand) was placed on a jack to allow vertical adjustment. A 1-cm hole was made at the center of the wok. A CCD camera (for micro-viewing) was mounted on a second stand which allow the CCD camera to be positioned to align with the hole. The CCD camera was connected to a 24" video-television so that
the reflected corneal image could be viewed from the screen and also recorded on videotape. A portable platform with a chin-rest attached was used to position the subject's head.

![Image of an eye with a grid pattern]

**Figure 4.4 Discontinuity (arrow) of the grid image.**

**Procedure (for NITBUT test)**

The subject was asked to sit comfortably with the arms resting on the table, forehead firmly pressed against the head-rest and chin resting on the chin-rest. The eye not under test was occluded. The subject fixated the hole in the center of the grid pattern with the test eye, and was asked to blink naturally a few times and then to keep the eyes open without consciously opening the eyes wider. S/he was required to look straight ahead and to refrain from blinking. However, s/he was permitted to blink when any discomfort was felt, to avoid reflex tearing. NITBUT was measured (see Figure 4.4) as either:

(a) the time from the last blink to the first appearance of a discontinuity in the grid image or

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(b) the time from the last blink to the first appearance of a distortion of the grid image, provided that the distortion then became a discontinuity.

Temporary distortion of the grid which does not become a discontinuity may be caused by debris moving in the tear film, causing disruption of the image. Distortion which resolved without leading to a break of the image were considered to be due to movement of debris and were disregarded. For each test, five to eight measurements were made for each subject. Measurements were made only after complete blinks. If the subject blinked before a discontinuity was observed in the tear film (blink-before-break), the measurement was repeated until five measurements were obtained. If there were more than three blink-before-break measurements, the test was terminated in order to avoid discomfort due to repeated measurements, and the result of this measurement was regarded as not measurable. The whole process was recorded on video.

4.2.2 SP-CTT

Material: Self-prepared cotton threads, millimeter rule, stopwatch.

Preparation of the cotton threads (Cho 1993a)

Based on the observation that colored threads tend to darken in color when wetted, a commercially available orange red cotton sewing thread (0.3 mm, brand '555', cotton thread made in China) was used for preparing the SP-CTT.

A 6 m length thread was folded six times (into 1 m lengths) and wound round a metal ring (diameter of about 12 cm). The thread was loosely attached to the ring, to prevent the thread from becoming loose during 30 min of boiling to remove the wax on the thread, and to loosen the thread for better capillary action.
The thread was removed from the ring and left to dry for about 1 hr. After drying, the thread was soaked in 90% alcohol for 3 hr and then dried overnight in a room of about 68% humidity and temperature of about 28°C. Each thread was cut into pieces about 70 mm long and stored in sterile plastic bags.

**Procedure (for SP-CTT)**

The subject was seated comfortably on the examination chair and asked to relax. The test and procedure was carefully explained to the subject and the subject was assured that the test was not painful. A prepared thread was removed from the sterile plastic bag and bent 5 mm from one end. The subject was asked to look up and nasally, and the lower lid was gently pulled down. The 5 mm bend was gently hooked over the lower lid margin at the temporal one-third and the lid gently released. A stopwatch was started immediately and the subject asked to maintain the position of gaze and to blink naturally. After 15 s, the lower lid was pulled
downward gently and the thread was removed. A notch was made with the fingernail immediately at the boundary of the wet portion. The wetted length of the thread appeared to be darker in colour (see Figure 4.5), and was measured from the notch to the tip with a millimeter rule. This value was recorded as the SP-CTT value in mm/15 s.

Figure 4.6 The Phenol red thread (PRT) test.

4.2.3 PRT test

Material: Zone-Quick Phenol red thread (Showa Yukuhin Kako Co. Ltd, Japan), millimeter rule, stop watch.

Procedure (for PRT test)

The measurement procedure was the same as the SP-CTT. When wetted with tears, the thread changed color from yellow to red (see Figure 4.6). The testing period and the unit of the value recorded were the same as for the SP-CTT test.
4.2.4 Subjective assessment of dry eye-related symptoms during contact lens wear

At the end of each season (see Section 7.1.2 - experiment on Effect of seasonal change on contact lens wear), each subject was asked to answer two questions related to the types and frequencies of any dry eye-related symptoms during contact lens wear. The questions were asked in written form in Chinese (see Appendix II). For presentation in this thesis, they were discussed in English (see Section 7.1.2).

4.2.5 Ocular examination using slit-lamp biomicroscope


Procedure

A thorough examination of the anterior eye (including the lid margins and lashes) using the slit lamp was performed on every subject at each visit, after the performance of the tear tests. Any complications observed were recorded carefully by drawing and description, and the Efron scale was used for grading where appropriate.

According to the Efron scale, the severity of the complications were graded as 0 (none), 1 (trace), 2 (mild), 3 (moderate) and 4 (severe). If the severity of a particular complication was found to be somewhere between two grades, it was recorded as the lower grade with the addition of a ‘+’ sign. For example, for hyperaemia more severe than mild but not as serious as moderate would be
recorded as 'hyperaemia - 2+'.

To examine corneal health, fluorescein from of a piece of fluorescein strip wetted by a drop of normal saline solution was gently applied to the superior bulbar conjunctiva while the subject was looking down. The subject was then asked to blink several times. The ocular surface of the subject was observed under low magnification with the aid of a cobalt-blue filter.

4.3 Subject Criteria

All subjects were HK-Chinese who wanted to wear contact lens and were recruited from one private practice. They all satisfied the following criteria unless otherwise stated:

a) good general and ocular health,

b) no contact lens wearing history,

c) not taking any medication,

d) aged from 16 to 40 years,

e) spherical Rx between −1.00 DS to −8.00 DS,

f) cylindrical Rx not more than −1.00 DC.

All subjects signed informed consent forms before participating in the experiments.

4.4 Ethical Approval

For each of the experiments reported in this thesis, ethical approval was obtained from the Ethics Sub-committee of The Hong Kong Polytechnic University.
Chapter 5
NITBUT, SP-CTT AND PRT VALUES AND THE EFFECT OF SOFT CONTACT LENS WEAR

It has been suggested that a contact lens placed on the corneal surface will compromise the normal functioning of the lacrimal system. Minor deficiencies of the lacrimal system, which would normally produce no symptoms, may result in poor tolerance to contact lenses (Holly and Lemp 1977, Fanti and Holly 1980). How does contact lens wear affect tear stability and tear volume? Investigators have not come to a consensus regarding this issue.

It has been reported that NITBUT decreased immediately after removal of a contact lens, followed by a recovery within a few minutes (Faber et al. 1991) (see Section 2.2.5 (viii)). Cho and Yap (1995) measured the change of NITBUT and SP-CTT before and after soft contact lens wear on 35 HK-Chinese. The baseline values of these tests were compared with values obtained after 2, 9 and 28 weeks of lens wear. They found that NITBUT values decreased after 2 and 9 weeks but not after 28 weeks of contact lens wear (See section 2.2.5 (viii)). They also reported no significant change in the SP-CTT value throughout the 28 weeks (See section 2.1.3 (ix)).

The aims of the present study are to determine the baseline values of the NITBUT test, SP-CTT and PRT test of HK-Chinese, and to confirm Cho and Yap’s results, that is, changes in these values with soft contact lens wear, using a larger sample. Data obtained from the right and left eyes are also compared.
5.1. Subjects and method

5.1.1 Subjects

Fifty-one Chinese (45 female, 6 male), of mean±SD age of 22.2±4.4 years, who wanted to wear contact lenses were recruited from a private practice in Hong Kong. All subjects satisfied the criteria stated in Section 4.3.

5.1.2 Method

Informed consent was obtained from each subject before the commencement of the study. All subjects were prescribed with daily wear Hydron Zero 6 (38% HEMA) soft contact lenses, and were asked to wear the lenses 14 hr a day, 5 - 6 days a week, after the adaptation period. The same lens care system, Allergan Oxysept B12, LC65 daily cleaner, Lensplus aerosol saline and Ultrazyme protein removal tablets, was prescribed for all patients. Lens fittings and data collections were all performed by the same examiner (WSC).

All subjects were required to attend a preliminary and lens fitting visit, a delivery visit, and at least four aftercare visits (after 1, 2, 9 and 28 weeks of lens wear). The delivery visit was denoted as week 0 and the tear tests were performed on weeks 0, 2, 9 and 28; the tear tests consisted of the NITBUT test for tear stability, and SP-CTT and PRT test for tear volume. The three tests were performed on both eyes of each subject. There was no systematic order with regard to which eye was tested first. The NITBUT test, due to its non-invasive nature, was always performed first. This was followed by the SP-CTT or the PRT test, in random sequence. This order was determined at the first visit, and remained the same for all visits for every subject. About half of the subjects had the SP-CTT performed after the NITBUT test, followed by the PRT test. The remaining subjects had the
PRT test performed after the NITBUT test and the SP-CTT was performed last. A resting interval of 10 min was allowed between tests. At weeks 2, 9 and 28, the tear tests were performed at least 15 min after lens removal. The procedures for all the three tests are described in Section 4.2.

At each visit, ocular examination was carried out as described in Section 4.2.5

5.2 Results

5.2.1 Right versus Left eye

The distribution of the NITBUT values was significantly different from a Normal distribution (Kolmogorov-Smirnov Test: p<0.05), while the distributions of both the SP-CTT and PRT values were not (Kolmogorov-Smirnov Tests: p>0.05). Hence, non-parametric statistical tests were used for NITBUT data, and parametric statistical tests were used for SP-CTT and PRT data.

Table 5.1 Comparison of NITBUT, SP-CTT and PRT values between the two eyes

<table>
<thead>
<tr>
<th>Eye</th>
<th>Median NITBUT (s)</th>
<th>Mean±SD SP-CTT (mm/15 s)</th>
<th>Mean±SD PRT (mm/15 s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>9.7</td>
<td>11.0±6.1</td>
<td>14.1±5.6</td>
</tr>
<tr>
<td>Left</td>
<td>9.3</td>
<td>9.7±6.1</td>
<td>13.4±6.1</td>
</tr>
</tbody>
</table>

*p=0.943
#p=0.131

*Probability value for Wilcoxon signed-rank test for differences in NITBUT values between the right and left eyes.

#Probability values for Paired t test for differences in SP-CTT or PRT values between the right and left eyes.

Table 5.1 shows the baseline results of the right and left eyes. Twelve subjects blinked before break (see Section 4.2.1) during the NITBUT measurements at week 0 on either or both eyes, therefore only the data from the remaining 39
subjects were analyzed. No significant differences were found between the two eyes for any of the three tests (NITBUT: Wilcoxon signed-rank test, \( Z=-0.72, p=0.943 \); SP-CTT: Paired t tests, \( p=0.131 \); PRT: Paired t tests, \( p=0.331 \)) (See Table 5.1). However, significant correlations were found between the two eyes (NITBUT: \( r=0.718, p=0.000 \); SP-CTT: \( r=0.457, p=0.001 \); PRT: \( r=0.541, p=0.000 \)).

### 5.2.2 Priority of eye

The right eyes were tested first and second on 23 and 28 subjects respectively. Since there were no significant differences between the right and left eyes for any test (See Section 5.2), only the data from the right eyes are analyzed here. One subject blinked before break in the group where the right eyes were tested first and 11 subjects blinked before break in the group having their right eyes tested second.

<table>
<thead>
<tr>
<th>Priority</th>
<th>Median (s)</th>
<th>Mean±SD (mm/15 s)</th>
<th>Mean±SD (mm/15 s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right eye tested first</td>
<td>11.9</td>
<td>11.1±6.8</td>
<td>13.4±5.7</td>
</tr>
<tr>
<td></td>
<td>( n=22 )</td>
<td>( n=23 )</td>
<td>( n=23 )</td>
</tr>
<tr>
<td>Right eye tested second</td>
<td>8.7</td>
<td>11.0±5.7</td>
<td>14.8±5.5</td>
</tr>
<tr>
<td></td>
<td>( n=17 )</td>
<td>( n=28 )</td>
<td>( n=28 )</td>
</tr>
<tr>
<td>*</td>
<td>( p^*=0.551 )</td>
<td>( p^#=0.519 )</td>
<td>#0.952</td>
</tr>
</tbody>
</table>

*Probability value for Mann-Whitney U test for difference in NITBUT between priority of eyes  

#Probability values for Unpaired t tests for differences in SP-CTT and PRT values between priority of eyes
No significant differences were found between the two groups in any of the three tests (NITBUT: Mann-Whitney U test, Z=-0.596, p=0.551; SP-CTT: Unpaired t tests, p=0.519; PRT: Unpaired t test, p=0.952) (See Table 5.2).

Table 5.3. Comparison of SP-CTT and PRT values for effects caused by priority of tests.

<table>
<thead>
<tr>
<th>Test priority (after NITBUT test)</th>
<th>Mean±SD SP-CTT value (mm/15 s)</th>
<th>Mean±SD PRT value (mm/15 s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP-CTT first</td>
<td>10.8±5.6</td>
<td>13.3±5.4</td>
</tr>
<tr>
<td>n=29</td>
<td></td>
<td>n=22</td>
</tr>
<tr>
<td>PRT first</td>
<td>11.4±6.9</td>
<td>15.2±5.7</td>
</tr>
<tr>
<td>n=22</td>
<td></td>
<td>n=29</td>
</tr>
<tr>
<td>p# = 0.485</td>
<td></td>
<td>p# = 0.958</td>
</tr>
</tbody>
</table>

p# - Probability values for Unpaired t tests for differences in SP-CTT and PRT values between priority of tests

Table 5.4. Changes in NITBUT, SP-CTT and PRT values from baseline values after different periods of contact lens wear.

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 2</th>
<th>Week 9</th>
<th>Week 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>NITBUT (n=18)</td>
<td>Median (s)</td>
<td>8.7</td>
<td>7.9</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p* = 0.136</td>
<td></td>
</tr>
<tr>
<td>SP-CTT value (n=39)</td>
<td>Mean±SD (mm/15 s)</td>
<td>11.6±6.5</td>
<td>10.3±5.5</td>
<td>10.9±6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p# = 0.296</td>
<td></td>
</tr>
<tr>
<td>PRT value (n=39)</td>
<td>Mean±SD (mm/15 s)</td>
<td>14.3±5.9</td>
<td>12.5±4.9</td>
<td>13.0±5.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p# = 0.300</td>
<td></td>
</tr>
</tbody>
</table>

* Probability values for Friedman test for differences between values obtained at different weeks.
# Probability values for Repeated Measures ANOVA (Dunnett Multiple Comparison (Post) Tests) for difference between subsequent weeks and Week 0 (baseline).
5.2.3 Priority of test (SP-CTT and PRT test)

SP-CTT was performed first (after NITBUT test) on 29 subjects while PRT test was performed first (after NITBUT test) on 22 subjects. Since there were no significant differences between the right and left eye for all three tests, only data from the right eyes were analyzed here.

There were no significant effects of priority of tests on the SP-CTT or PRT values (SP-CTT: Unpaired t test, p=0.485; PRT: Unpaired t test, p=0.958) (see Table 5.3).

5.2.4 Effect of soft contact lens wear on NITBUT, SP-CTT and PRT values

Of the 51 subjects, 12 subjects did not show up for measurements in one or more subsequent visits. Therefore only 39 subjects were used for comparison between visits. Of these 39 subjects, twenty-one subjects blinked before break in one or more visits during the NITBUT measurements of the right eye. Therefore only the data from 18 subjects were available for comparison of NITBUT values between visits. For the SP-CTT and PRT test, data of the 39 subjects who attended all visits were included for analysis. Friedman test was used for comparison of NITBUT values and the Repeated measures ANOVA, with Dunnett Multiple Comparison (Post) Tests were used for SP-CTT and PRT values.

Table 5.4 shows a summary of the results of the statistical analysis. The median baseline NITBUT was 8.7 s (n=18). The baseline SP-CTT values ranged from 3 to 31 mm/15 s and the mean±SD value was 11.6±6.5 mm/15 s (n=39). The baseline PRT value ranged from 5.5 to 30 mm/15 s and the mean±SD value was 14.3±5.9 mm/15 s (n=39). No significant changes from the baseline values were observed in any of the three tests (NITBUT, p=0.136; SP-CTT, p=0.296; PRT, p=0.300).
5.3 Discussion

There were no significant differences in the NITBUT, SP-CTT and PRT values between the right and left eyes, and the priority of the eye being tested first made no significant difference to the result. Performing the SP-CTT first did not significantly affect the PRT result and vice versa, if a 10 min rest was allowed between tests.

The NITBUT, PRT and SP-CTT values, taken after lens removal, were not significantly affected by soft contact lens wear. Our results on the PRT and the SP-CTT value were in consensus with Cho and Yap (1995) (see Section 2.1.3 (ix)). However, our results on NITBUT values did not quite agree with their finding (see Section 2.2.5 (viii)). In their study, they observed an initial decrease (at weeks 2 and 9) in NITBUT value with the commencement of contact lens wear, but this change was transient and self-correcting. In our study, there was no significant decrease (from baseline) in NITBUT value at weeks 2 or 9.

It was not uncommon to find subjects with NITBUT values longer than 1 min. To perform five to seven NITBUT measurements each time on each eye (see Section 4.2.1), in addition to a series of other clinical tests and procedures, as in our study, was time consuming and rather tiring for the subjects. Measuring one eye only would greatly simplify the experimental procedures and would save time for both the examiner and the subjects. Our results showed no significant difference between the two eyes for any of the three tests used (See Section 5.2.1), and there were good correlations of measurements between the two eyes. Hence, in the following studies (Chapter 6 and 7), we performed these tear tests on one eye only on the new subjects. Our results are in consensus with reports by previous investigators, that there are no significant differences in the tear stability (Tiffany
et al. 1989, Tonge et al. 1991) and tear volume (Kurihashi 1986, Cho and Yap 1995) between the right and left eye of a subject, and measurements from the two eyes are strongly correlated. Hence, data from the two eyes of a subject should not be treated as independent data (see Section 2.2.7)

Our results indicated that performing the SP-CTT before the PRT test did not significantly affect the PRT values and vice versa, provided that there was a 10 min rest interval between the two tests. Cho and Douthwaite (1994) reported that 10 min was enough for the tear volume to return to baseline after a SP-CTT measurement with testing period of 1 min. In the current study, we used two different types of cotton thread tests, the SP-CTT and the PRT test, to measure tear volume. These two tests, although not equivalent, uses the same principle with respect to tear absorption and measurement technique. It has been reported that 1 μl of fluid is enough to wet 25 mm and 33 mm of the threads used in SP-CTT and PRT test respectively (Cho and Kwong 1996). The ratio of SP-CTT to PRT values was 0.76. The ratio of the mean SP-CTT to PRT wet lengths obtained in the current study was comparable to this ratio (See Tables 5.1, 5.2 and 5.3). The mean SP-CTT value obtained in the current study was less than the value reported by Cho and Douthwaite (1994) as they used 1 min testing period instead of 15 s. The invasiveness of the SP-CTT and PRT test, with a testing period of 15 s, is minimal so the tests are unlikely to affect subsequent tear assessments. A resting interval of 10 min may be more than enough for recovery if a testing period of 15 s is used instead of 1 min. Further investigation is required in this area.

Cho and Yap (1995) reported an initial transient reduction of NITBUT after commencing contact lens wear. A similar trend of change of TBUT was reported by Kempster et al. (1979). They found that the TBUT initially decreased after one
week of lens wear and it tended to return to baseline after one month of lens wear. However, these changes were not statistically significant. In our study, the median NITBUT values were lower in week 2 and week 9, but the differences were not significant (see Table 5.4). However, the sample size for analysis of the change of NITBUT values between visits was rather small (n=18), since the data of a large number of subjects were excluded due to blink-before-break cases and/or not returning for one or more of the tear test visits. This greatly limited the power of our analysis since the variability of the NITBUT value was comparatively large.

Kline and DeLuca (1975), in a report on ST suggested that contact lenses interfere with the mechanism by which mucin is spread over the cornea and perhaps disrupts the lipid layer of the tear film, which in turn increases the evaporation of tears. It appears that the human eye has the ability to compensate for such changes. Our results agreed with the studies reported by Cho and Yap (1995) and Hamano (1983) that tear volume, as measured by either SP-CTT or PRT test, are unaffected by soft contact lens wear. Future studies comparing the difference between the tear volume of successful and unsuccessful contact lens wearers may recruit existing contact lens wearers as subjects to facilitate recruitment of larger subject samples.

5.4 Conclusion

NITBUT, SP-CTT and PRT values are not significantly different between the two eyes, and test values between the right and the left eyes are highly correlated as expected. Priority of the eye to be tested first does not affect the result. Performing the SP-CTT or PRT test first does not affect the values of subsequent PRT test or SP-CTT respectively, if a 10 min resting interval is allowed between
tests.

Our study confirms that soft contact lens wear has no effect on tear volume (measured after lens removal). Tear stability (after lens removal) is shown to be constant after soft contact lens wear, but because of the low statistical power of the test used, this finding is felt to be inconclusive.
Chapter 6

PREDICTING SUCCESS OF SOFT CONTACT LENS WEAR USING THE NITBUT, SP-CTT AND PRT TESTS

Cho and Yap (1995) reported that the SP-CTT appeared to be more useful than the NITBUT test in differentiating soft contact lens wearers with or without contact lens problems related to dry eye. However, in their study, there were only two subjects classified as having problems. Also, during the period of their study, the PRT was not commercially available so they did not use the PRT test. The aims of the current study were to confirm the usefulness of NITBUT, SP-CTT and PRT tests in predicting successful soft contact lens wear. We also investigated the usefulness of wetting values (Wv), the combination of NITBUT and SP-CTT values, or NITBUT and PRT values, for predicting successful soft contact lens wear.

6.1 Subjects and method

6.1.1 Subjects

All the 51 subjects enrolled in the experiment described in Chapter 5 were included in this study, and we recruited 30 more subjects who also satisfied the criteria listed in Section 4.3. Of these extra 30 subjects, three subjects were excluded for various reasons (see Section 6.2). Therefore, only the data of 78 subjects (67 females, 11 males), of mean±SD age of 21.6±4.1, was included.
6.1.2 Method

6.1.2 (i) Procedure

For the 51 subjects who participated in the experiment described in Chapter 5, we used the baseline values of their right eyes (if available). Twelve of these 51 subjects blinked before break during the measurement of NITBUT on the right eye, but six of them had measurable results for the left eye. In order to maximize our sample numbers, the results for the left eye for all three tests were used for these six subjects. For the newly recruited 30 subjects, the three tear tests were performed on the right eye at week 0 (before commencing soft contact lens wear) only using the same procedures described in Section 5.1.2.

This experiment was run in conjunction with the experiment described in Chapter 5. At each aftercare visit, at weeks 1, 2, 9 and 28, the subjects were asked to report any symptoms related to dry eye (such as soreness, scratchiness, dryness, grittiness, burning etc.), during lens wear and after lens removal. Wearing times were recorded. Slit lamp examination was carried out at each visit, and ocular signs, such as inferior mid-peripheral corneal staining, interpalpebral conjunctival staining or hyperemia, were recorded and graded where applicable (see Section 4.2.5).

6.1.2 (ii) Combination of tests

In this study, we defined two new wetting values using the following equations,

\[ W_{VP} = f_1 (\text{NITBUT}) + f_2 (\text{PRT}) \]
\[ W_{VS} = f_1 (\text{NITBUT}) + f_2 (\text{SP-CTT}) \]

where \( f_1 \) and \( f_2 \) were 1 \( s^{-1} \) and 1 \( (\text{mm/15 s})^{-1} \) respectively (see Section 2.3).
6.1.2 (iii) Classification of subjects in terms of signs, symptoms and wearing time

After 28 weeks of contact lens wear, the subjects were separated into two groups. Group 1 subjects were successful wearers, who achieved 12 hr or more of lens wear with no signs or symptoms. Group 2 subjects were unsuccessful wearers, subjects who had signs and symptoms, even when their daily wearing times were reduced to 12 hr or less.

6.2 Results

Three subjects failed to complete the study: one left the study due to personal reason, the other due to non-compliance, and the third could not build up her wearing time. Their data were excluded, and therefore only the data from 78 subjects are presented here.

6.2.1 Distribution

As the distribution of the NITBUT values was significantly different from Normal, non-parametric statistical tests were used for NITBUT data (see Section 5.2.1). The distributions of both SP-CTT and PRT values were not significantly different from Normal, so parametric tests were used for SP-CTT and PRT data (see Section 5.2.1). For Wvs and Wvp, non-parametric tests were used.

6.2.2 Baseline value

For NITBUT, eight subjects blinked before break during NITBUT measurement, so only data from 70 subjects were analyzed. The median NITBUT was 8.7 s. The SP-CTT value (n=78) ranged from 3 to 31 mm/15 s and the mean±SD value was 10.6±6.3 mm/15 s. The PRT value (n=78) ranged from 4 to 30 mm/15 s and the
mean±SD value was 13.9±5.6 mm/15 s.

Table 6.1. Baseline values of Groups 1 (successful wearers) and 2 (unsuccessful wearers) subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of subjects</th>
<th>mean±SD SP-CTT (mm/15 s)</th>
<th>mean±SD PRT (mm/15 s)</th>
<th>median NITBUT (s)</th>
<th>Wvs median</th>
<th>Wvp median</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>12.9±7.5</td>
<td>15.2±6.0</td>
<td>8.7</td>
<td>25.4</td>
<td>26.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*n=30</td>
<td>*n=30</td>
<td>*n=30</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>8.8±4.6</td>
<td>12.9±5.2</td>
<td>11.4</td>
<td>19.5</td>
<td>26.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*n=28</td>
<td>*n=28</td>
<td>*n=28</td>
</tr>
</tbody>
</table>

SP-CTT - Self prepared-cotton thread test
PRT - Phenol red thread
NITBUT - Non-invasive tear break-up time
Wvs - wetting value defined by NITBUT and SP-CTT value
Wvp - wetting value defined by NITBUT and PRT value
*Number of subjects with measurable NITBUT value

6.2.3 Group difference

Subjects who had mild to moderate signs and any symptoms of dry eye during lens wear were mainly advised to reduce their wearing time rather than given other treatments. Those who had signs and symptoms of dry eye which persisted even after reducing the wearing time, and those who showed severe corneal desiccation or complications other than dry eye, such as solution-related sensitivity, ocular allergy...etc., were treated accordingly as described below. Three of these subjects continued to have problems even after reducing the wearing time to <10 hr per day after week 9. They were allowed to use eye drops (Opti-tears - Alcon), for no more than 4X a day, to relieve their symptoms. One subject reported obvious dryness, and dense inferior punctate corneal staining was found. This subject left the study after week 9 and was classified under Group 2.
(see Section 6.1.2 (iii)) although she did not complete the study. One subject developed superior epithelial arcuate lesion on both eyes. This subject was refitted with Weicon 38E soft lenses, which is also a 38% HEMA lens, and with center thickness comparable to Hydron Zero 6. The lesion soon regressed after refitting, and her data was included for analysis. None of the subjects showed any other contact lens-related complications.

Eight subjects had signs only and four subjects had symptoms only. Since they did not satisfy the criteria for Group 2 (see Section 6.1.2 (iii)), data from these subjects were discarded.

Table 6.1 shows a summary of the results obtained for the remaining 66 subjects. Thirty-four subjects and thirty-two subjects were classified as Group 1 (successful wearers) and Group 2 (unsuccessful wearers) respectively (see Section 6.1.2 (iii)). No significant difference in the baseline NITBUT was found between the two groups (Mann-Whitney U test: \( Z = -1.348, p = 0.178 \)). The mean baseline SP-CTT value of Group 1 subjects was significantly longer than that of Group 2 subjects (Unpaired t test: \( p = 0.002 \)). Although the mean baseline PRT value of Group 1 was longer than that of Group 2, the difference was not statistically significant (Unpaired t test: \( p = 0.358 \)).

The Mann-Whitney U test showed no significant difference in the Wvs and Wvp between the two groups of subjects (Wvs: \( Z = -1.401, p = 0.161 \); Wvp: \( Z = -0.405, p = 0.686 \)).

6.2.4 Evaluation of the tests used

Since the baseline NITBUT, PRT, Wvs and Wvp were not significantly different between Group 1 and Group 2 subjects, we calculated the specificity, sensitivity,
the positive and negative predictive values of the SP-CTT only.

Positive predictive value refers to the probability of unsuccessful contact lens wearers among those with positive test results. Negative predictive value refers to the probability of successful contact lens wearers among those with negative test results (Sorbara and Talsky 1988).

![Chart showing sensitivity and specificity](image)

**Figure 6.1 Accuracy of the SP-CTT.**

The sensitivity and specificity of SP-CTT were determined using different cut-off values (0.5 steps) to determine the values which gave the optimal sensitivity and specificity in predicting contact lens-induced dry eye problems (see Figure 6.1). The sensitivity and specificity values for the SP-CTT were 62.5% and 61.8% respectively, when the cut-off value of 9 mm/15 s was used. The positive and negative predictive values at this cut-off point were 60.6% and 63.6% respectively. However, if we increased the cut-off value to 12.5 mm/15 s, the sensitivity increased to 81.3% but the specificity only decreased by about 6% (to
55.9%), and the positive and negative predicting values were both increased to 63.4% and 76.0% respectively.

6.3 Discussion

The results of this study confirmed Cho and Yap’s (1995) suggestion that baseline SP-CTT value was more useful in predicting successful soft contact lens wear while baseline NITBUT value was not. Baseline PRT value, Wvs and Wvp were also poor predictors of successful soft contact lens wear. The sensitivity and specificity of the SP-CTT, using 12.5 mm/15 s as the cut-off value, were 81.3% and 55.9% respectively while the positive and negative predictive values were 63.4% and 76.0% respectively.

Tonge et al. (1991) suggested that contact lens wearers with NITBUT <20 s should be carefully monitored. Our results showed that the NITBUT test was of little value in the identification of patients who were likely to be unsuccessful in contact lens wear. If we used 20 s as a cut-off value, our NITBUT result yielded a sensitivity of 71.4%, but the specificity was only about 20%. Mengher et al. (1986a) reported that NITBUT values provided a sensitivity of 82% and specificity of 86% in diagnosing dry eyes when a critical value of <10 s was used. However, in their study, both eyes of each subject were measured and these data were regarded as independent; hence the validity of their result is questionable on statistical grounds (see Section 2.2.7). In any case, the critical value suggested by Mengher et al. would not be applicable to HK-Chinese as over 50% of the subjects in the current study had baseline NITBUT of <10 s. This is in agreement with previous reports on HK-Chinese (Cho and Yap 1995).

Little and Bruce (1994) proposed diagnostic criteria of <11 mm/15 s and <16
mm/15 s for low and borderline tear volume respectively after measuring the PRT value of the right eyes of 17 asymptomatic subjects. In our study, among our Group 1 and Group 2 subjects (n=66), 20 subjects had baseline PRT values of <11 mm/15 s, and nine of these subjects could wear their lenses without any signs and symptoms of dry eye. All our subjects were asymptomatic before contact lens wear, so it is possible that the subjects classified into the unsuccessful wearers group were marginal dry eye patients. If we used the borderline tear volume criterion of <16 mm/15 s as the cut-off value, as suggested by Little and Bruce (1994), the PRT test provided a sensitivity of 65.6% and a specificity of 47.1% for our subjects.

Hamano et al. (1983) reported that 58% of their subjects wearing soft contact lenses and with a PRT value <9 mm/15 s had subjective dry eye symptoms. In our study, 15 subjects had baseline PRT values <9 mm/15 s but only 10 (66.7%) were classified as unsuccessful wearers. Hamano et al. (1983) did not calculate the specificity and sensitivity of the PRT test on their Japanese subjects. If we used 9 mm/15 s as the cut-off value in our study, the sensitivity of the PRT test was only 31.3% while the specificity was 85.3%; and the positive and negative predictive values were 66.7% and 56.9% respectively. We found that the mean baseline PRT value of the successful lens wearers was not significantly higher than the unsuccessful lens wearers. Kwong and Cho (1998) have also reported that this test cannot be used to differentiate normal from dry eye subjects.

In the current study, the ratio of the mean baseline SP-CTT value and PRT value was 0.76 which was in close agreement with previous reports (Cho and Kwong 1996 - 0.76; Kwong and Cho 1998 - 0.73). Using this ratio as a guideline, 16 mm of PRT value (borderline tear volume suggested by Little and Bruce (1994)) is
equivalent to 12 mm of SP-CTT value which is close to the critical value (12.5 mm/15 s) found in our study, yielding a sensitivity and specificity of 81.3% and 55.9% respectively. If we use a PRT value of <9 mm/15 s as suggested by Hamano et al. (1983), the approximately equivalent of SP-CTT value was 7 mm. Using this cut-off value, the specificity of SP-CTT was 70.6% and the sensitivity was only 40.1%. The cut-off value for SP-CTT determined directly from the PRT cut-off value suggested by Hamano et al. (1983) was not suitable for differentiating the problematic soft contact lens wearers in the Chinese population. Although the PRT test is specially designed for the assessment of tear volume, it is not as predictive as the SP-CTT for our subjects. In a recent study, Kwong and Cho (1998) found that the SP-CTT performed better than the PRT test in the diagnosis of dry eye. We are not sure why this is so. Possible reasons may be related to the capacities and the physical properties of the threads.

Table 6.2. The amount of tears absorbed by the threads estimated from the baseline mean values in the PRT test and the SP-CTT of Groups 1 (successful wearers) and 2 (unsuccessful wearers) subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tear volume absorbed by the SP-CTT thread (μm)</th>
<th>Tear volume absorbed by PRT (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.52</td>
<td>0.46</td>
</tr>
<tr>
<td>2</td>
<td>0.35</td>
<td>0.39</td>
</tr>
</tbody>
</table>

SP-CTT - Self prepared-cotton thread test
PRT - Phenol red thread

In a pilot study (unpublished data), the wet length created by 1 μm of saline solution for the PRT and the SP-CTT were 33 mm and 25 mm respectively. Using these proportions, we may estimate the amount of tear absorbed by the PRT and
SP-CTT threads in successful and unsuccessful wearers (see Table 6.2) from the results shown in Table 6.1. The tear volume of successful wearers is presumably greater than that of unsuccessful wearers. Hence, it is possible that the maximum capacity of the PRT had already been reached or exceeded when used for absorbing tears of successful wearers. On the contrary, the capacity of the self-prepared cotton thread is comparatively higher. Hence, the SP-CTT was able to show the difference between normal and reduced tear volume, while the PRT test was not.

It is also possible that the phenol red dye affects the absorption of tears in different subjects. Our self-prepared cotton threads were purified by boiling in water. Most of the water soluble substances were therefore removed. The dye remaining in the thread was non-ionic and relatively non-hydrophilic. Hence, the driving force of fluid absorption was presumably the capillary action within the fibres of the thread only. However, for the PRT, phenol red dye which is water-soluble is added to the purified threads, so fluid absorption may be achieved by factors other than capillary action. Inter-subject difference in tear constituents may affect the absorption rate of the tear fluid. This creates an extra variable other than the tear volume which can affect the wet length of the thread. It is possible that the PRT test was more prone to be affected by the tear quality of the subject. Further investigation is required in this area.

Our results confirmed that only the SP-CTT can predict successful soft contact lens wear. It appeared that as long as the tear volume is adequate to maintain the hydration of the contact lenses in vivo, tear stability as measured by the NITBUT test is unimportant. The overall accuracy of the SP-CTT was not as high as we hoped, but it could be used as a screening test. From a clinical point of view, the
positive and negative predictive values are of greater value. Using a cut-off value of 12.5 mm/15 s, the positive and negative predictive values of the SP-CTT were 63.4% and 76.0% respectively. Patients with low SP-CTT baseline value should be carefully monitored. Prophylactic advice, such as strict compliance of wearing time and lens care regimen, awareness of blinking and avoidance of provocative environments, should be given. The chances of success can be explained to these patients, so that they can avoid unrealistic expectations.

Wetting values, using combination of NITBUT and PRT values, and NITBUT and SP-CTT values, do not predict successful soft contact lens wear. This was probably due to the large variability of NITBUT values (Cho 1993b, Brown and Cho 1994).

6.4 Further investigation

Further investigation can be concentrated on the efficacy of the SP-CTT on identifying contact lens wearers with dry eye problems. A large scale study may be conducted where the subjects may be recruited from the files of existing contact lens wearers in large clinics such as the clinic in an optometry school, or in a number of private practices. The subjects can be classified into different levels of success in contact lens wear according to the severity of signs and symptoms (if any) related to dry eye, and the periods of lens wear that do not cause dry eye symptoms reported by the patients. The SP-CTT can be performed on each subject with the examiner masked as to which class the subject belongs to. The large population of patients available, assuming adequate documentation, and the simple testing procedure greatly facilitated the process of subject recruitment and data collection. Subjects can also be classified into groups, in
terms of types of lens wear, lens care systems, age and gender, working environment and job nature. This allows better control of different factors that may affect the results.

It would also be interesting to investigate the efficacy of different types of dry eye management methods which have been suggested to be able to increase the tear volume (see Section 8.2). Most practitioners prescribe tear supplement to treat dry eye problems. Some of these eye-drops contain a muco-mimic substance and are claimed to be able to be retained longer in the eye. Some of them are preservative free (single-dose) and allow more frequent application without being compromised by the side effects of the preservative. The newest multi-dose systems, preserved with fast depleting preservatives, are also available - these preservatives decompose soon after exposure to light. To compare the efficiency of these eye-drops, several groups of soft lens wearers with comparable but below critical cut-off SP-CTT value may be recruited. Each subject group would be prescribed with different types of eye-drops while one of these subject groups should receive placebo treatment to serve as control. The percentage of successful wearers and the occurrence of signs and symptoms related to dry eye of each treatment group can be compared with the control group. The effectiveness of different dry eye management methods may also be investigated in the same manner.

Another aspect for further study may be the effect of tear viscosity and tear protein level on the wet length of the SP-CTT thread and the PRT. This can help us to determine if the poor sensitivity of the PRT test is due to the tear quality as the PRT thread may be more prone to be affected by the constituents of the tears (see Section 6.3). Artificial tear samples of different protein concentrations can be
prepared using physiological saline and standard proteins. The samples can also be prepared with different viscosity by mixing saline with different amounts of muco-mimic agent. One end of the SP-CTT thread and PRT can be dipped into the 'tear' samples. The correlation between protein concentrations and the viscosity of the 'tear' samples and the wet lengths of the threads can be then investigated.

6.5 Conclusion

Our study showed that the NITBUT and the PRT tests were not clinically useful in screening potential soft contact lens wearers. Only the SP-CTT test can predict the chance of successful soft contact lens wear with moderate sensitivity, specificity, and positive and negative predictive values. Wetting values, using combinations of NITBUT and SP-CTT or PRT values cannot be used to predict success in soft contact lens wear.

Paper published:
Chapter 7

EFFECT OF SEASONAL CHANGE ON SOFT CONTACT LENS WEAR

It has been reported that dry eye symptoms can be provoked by environmental factors such as in smoky or low humidity environments (Basu et al. 1978, Shapiro and Merin 1979, Nilsson and Andersson 1986). Anecdotal evidence has also indicated that some patients have more problems with their contact lens wear in dry weather. In Hong Kong, the humidity is generally low in autumn and winter (starting around October), and high in spring and summer (starting around February). Since the main part of our study (Chapters 5 and 6) involved changes of tear functions and the signs and symptoms during soft contact lens wear over a period of more than half a year, therefore, the study was carried out through different seasons. It is important to know whether seasonal change in HK can influence subjects’ signs and symptoms during contact lens wear. It is also important to know whether the change in humidity can cause changes in the quantity and quality of the tears since it is well documented that these factors can play important roles in determining the success of contact lens wear (Holly 1981b; Sharma and Ruckenstein 1985; McMonnies 1990; White 1993).

The aim of this experiment was therefore to determine if humidity changes due to seasonal changes in HK affected the signs and symptoms, the NITBUT, SP-CTT and PRT values of HK soft contact lens wearers. This was to ensure that results in the main part of our study (Chapters 5 and 6) were not affected by the change in season.
7.1 Subjects and method

7.1.1 Subjects

All subjects who had completed the study described in Chapter 6, except those who were allowed to use eye drops (see Section 6.2.3), were invited to take part in this experiment, but only 15 subjects agreed to participate.

7.1.2 Method

All subjects had about six months of soft contact lens wear history. They were all wearing the same lens type, and using Allergan Oxysept lens care system (see Section 5.1.2). For each patient, a new pair of soft contact lenses were provided during the period of mid October to early November (dry season), and the NITBUT, SP-CTT and PRT values were measured. All the subjects were asked to maintain their cleaning regimens and their habitual wearing times and schedules.

An aftercare visit was required one month after wearing the new lenses. Each subject was asked to respond to two questions related to dry eye during soft contact lens wear (see Appendix II). The first question required the subjects to select the symptom(s), if any, which they had experienced in the last month. They were given five options: Soreness, Scratchiness, Dryness, Grittiness and Burning. The second question asked the subjects to indicate the frequency of having the symptom(s) referring to the first question: 0 (never), 1 (sometimes), 2 (often) and 3 (constantly). Ocular examination with the slit lamp was carried out as described in Section 4.2.5. Each subject was examined carefully for the presence of corneal staining. The soft lenses worn by each subject were replaced with a new pair in the month of March and April (humid season), and the same procedures were repeated. The data obtained during the two seasons were compared. The daily
relative humidity during the dry and humid seasons were obtained from the Hong Kong Observatory.

![Graph showing humidity distribution](image)

**Figure 7.1** The distribution of humidity in Hong Kong over the dry and humid seasons during the study period.

### 7.2 Results

The mean±SD relative humidity of the dry season (mid-October to mid-December) and the humid season (mid-April to early June) were 70.4±12.1% and 85.1±4.9% respectively. There was a significant difference in the relative humidity between the two seasons (Unpaired t test p<0.05) (see Figure 7.1).

<table>
<thead>
<tr>
<th>Table 7.1 Summary of results obtained in the dry and humid seasons.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median NITIBUT (s)</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Mean±SD SP-CTT value (mm/15 s)</strong></td>
</tr>
<tr>
<td><strong>Mean±SD PRT value (mm/15 s)</strong></td>
</tr>
<tr>
<td><strong>Median/mode Efron score of corneal staining</strong></td>
</tr>
</tbody>
</table>
Table 7.2 The number of subjects having a particular dry eye symptom.

<table>
<thead>
<tr>
<th>Season</th>
<th>Dry eye symptoms</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soreness</td>
<td>Scratchiness</td>
<td>Dryness</td>
<td>Grittiness</td>
<td>Burning</td>
</tr>
<tr>
<td>Dry</td>
<td>0</td>
<td>5</td>
<td>11</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Humid</td>
<td>0</td>
<td>4</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 7.1 shows a summary of the results obtained. For the three tear tests, there were no significant differences in the values obtained between the two seasons. (NITBUT: Wilcoxon signed-rank tests: $Z=−1.364$, $p>0.05$; SP-CTT: Paired t test: $p>0.05$; PRT: Paired t test: $p>0.05$). There were no significant differences in the degree of corneal staining observed between the two seasons (Wilcoxon signed-rank tests: $Z=−1.308$, $p>0.05$).

For each of the five symptoms, there was no significant difference in the percentages of subjects who reported the symptom between the two seasons (Chi square: $p>0.05$) (see Table 7.2).

Table 7.3 Frequency and severity of symptoms.

<table>
<thead>
<tr>
<th>Season</th>
<th>Frequency of experiencing the symptoms</th>
<th>Number of symptoms (median/mode)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Sometimes</td>
</tr>
<tr>
<td>Dry</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Humid</td>
<td>2</td>
<td>13</td>
</tr>
</tbody>
</table>

The number of symptoms selected by the subjects were compared between the two seasons, and no significant difference was found (Wilcoxon signed-Rank tests: $Z=−0.378$, $p>0.05$). There was no significant difference in the frequency of occurrence symptoms between the dry and humid seasons (Wilcoxon Signed Rank test: $Z=−1.000$, $p=0.05$) (see Table 7.3).
7.3 Discussion

Our results indicated that seasonal change in humidity in HK during the period of this experimental study did not affect the NITBUT, SP-CTT and PRT values, and dry eye-related signs and symptoms during soft contact lens wear, though we are aware of the limited power of these analysis due to small sample size.

The subjects in our study did not show any significant difference in the number and occurrence of dry eye-related symptoms and corneal staining between the two seasons. This may be because the difference in relative humidity of HK during the period of this study was not large enough to cause any difference in signs and symptoms. However, it should be noted that the examination room humidity was fairly stable. The ambient humidity may somehow affect the level of staining, but the symptoms reported by the subjects (during the last month) should not be affected. A point to note though was that during waking hours, when they were wearing their lenses, the subjects usually spent their time indoors, in offices, where the relative humidity was relatively consistent in spite of the humidity changes due to the weather. Hence, our results were not actually looking at effect of humidity changes per se.

The location of the corneal staining observed in our subjects was either inferior cornea or in the inferior mid-peripheral cornea, a typical sign of ocular desiccation. All staining observed was very mild. The majority of the subjects either had no staining in both seasons, or had some trace staining in both seasons. Some of the subjects (n=3) had staining in the dry season but not the humid season and vice versa in some other subjects (n=3). Bickel and Barr (1997) found that the corneal integrity was not compromised by variations of ambient humidity, but Osborn and Zantos (1988) reported that corneal staining was more likely to
occur when the ambient humidity was low. In the current study, the ambient humidity of the examination room was about 70%.

Bickel and Barr (1997) compared the comfort and corneal staining of 11 rigid gas permeable (RGP) contact lens wearers inside a modified examination room with variable ambient humidity on two separate days. The original ambient humidity of the room was 40%. On the first day, the ambient humidity was reduced to a range of 22% - 37% for two hours. The subjects were asked to rank the level of lens wear comfort and the corneal integrity was checked before and after the testing session. On the second day, the same procedures were repeated, except that the ambient humidity was elevated to a range of 56% - 63%. No significant difference in the severity of corneal staining was found at the end of each session when compared to the beginning of the testing sessions. In the lower humidity session, three subjects reported reduced comfort, one subject reported improved comfort and the remaining subjects reported no difference in comfort. In the higher humidity session, one subject reported improved comfort and nine subjects reported no difference in comfort. Bickel and Barr (1997) reported that there was a trend of decreasing comfort for RGP contact lens wear in a lower humidity environment.

Orsborn and Zantos (1988) studied the effect of humidity and corneal staining on four soft lens wearers who were wearing thin (0.04 mm), high water content (70%) contact lens. In the first phase, the subjects wore the lenses for two hours in an office of approximately 20% relative humidity. In the second phase, the subjects were transferred to a small room with the humidity controlled at approximately 80% for two hours. In the third phase, the subjects were returned to the original office for another two hours. At the end of each study phase, the
lenses were briefly removed and the corneal staining was assessed. They reported that all four patients developed moderate to dense confluent staining and discomfort in their eyes in the first phase. However, the discomfort soon disappeared after the subjects entered the humid room and the staining “practically” disappeared at the end of the second phase. In the third phase, the corneal staining and discomfort returned again. They suggested that, “1) desiccation staining is more likely to occur when the ambient humidity is low; 2) the staining can reverse quite quickly; 3) patient discomfort is correlated with low ambient humidity when wearing thin high water content lenses”. However, since they had only examined the staining at 20% relative humidity for the low humidity level, it is unclear how they came into the third conclusion.

Nilsson and Andersson (1986) compared the relative humidity of the working places of 11 soft lens wearers who were asymptomatic with those of 23 soft lens wearers who complained of discomfort during working hours. The mean±SD relative humidity of the working places of these two group of subjects were found to be 34.2±2.7% and 27.4±1.5% respectively, and the difference was statistically significant. They also investigated the level of discomfort of 10 subjects, five with TBUT <20 s, and five with TBUT>20 s, after staying for >3 hr in a room where the humidity was varied (to and fro) between 18% - 52%. They found that the level of discomfort of the former group followed the level of humidity “quite well – with some delay”, whereas the latter group showed much less variation. They concluded that the subjects with shorter TBUT were much more sensitive to a low relative humidity than the subjects with longer TBUT. However, they did not specify the types of work that the subjects were performing in their working places, which may somehow affect the level of discomfort.
Previous studies have reported that contact lens wear in low humidity environments resulted in more dry eye symptoms (Nilsson and Andersson 1986, Bickel and Barr 1997) and corneal staining (Orsborn and Zantos 1988). However, the effect reported by these investigators were rather short term, whereas the seasonal change investigated in the current study was over a much longer period.

A number of reported studies (Nilsson and Andersson 1986, Bickel and Barr 1997) compared the dry eye symptoms during contact lens wear by asking their subjects to rank the severity of discomfort of lens wear under environments with different ambient humidities. We did not do that because the severity of symptoms may be indicated by the number of symptoms options and the symptoms frequency selected by a patient (McMonnies 1986). In the current study, the results of the two questions about symptoms during soft lens wear did not show any significant difference between the two seasons, which agreed with our tear test results. Symptoms experienced by our subjects were unaffected by the change in humidity in the seasons.

7.4 Conclusion

Within the constraints of relatively small sample, our results demonstrated that the seasonal change in humidity in HK did not affect the NITBUT, SP-CTT and the PRT values of our subjects. The signs and symptoms related to dry eye during soft contact lens wear were also unaffected by the seasonal change. Thus we may assume that the results of our main study on the effect of contact lens wear on tear stability and tear volume (Chapter 5), and prediction of success of contact lens wear (Chapter 6) were unlikely to be affected by the seasonal change during the experimental study.
The data in this Chapter was presented in the 12th Asia-Pacific Optometric Congress, Manila, 14-18 March, 1999.
8.1 Summary of current study

There were strong and significant correlations of NITBUT, SP-CTT and PRT values between the right and left eyes. We confirmed that the tear volume as measured by the SP-CTT and PRT test after lens removal was not affected by soft contact lens wear. Cho and Yap (1995) reported an initial decrease in the NITBUT value at the commencement of soft contact lens wear. However, we did not find any significantly change in the NITBUT value (measured after lens removal) after commencement of soft contact lens wear.

Our results were in agreement with Cho and coworkers’ reports that the median NITBUT value (Brown and Cho 1994) and the mean SP-CTT and PRT values (Cho and Douthwaite 1994) were all lower than previous reports using Caucasian subjects. The diagnostic criteria for pathological dry eye suggested by previous reports were not applicable to HK-Chinese subjects used in our study since a number of asymptomatic subjects had test values below the norms suggested in those criteria.

Among the three tear tests, only the SP-CTT was able to predict the chance of dry eye problems during soft contact lens wear. When a cut-off value of 12.5 mm/15 s was used, the sensitivity and specificity were 81.3% and 55.9% respectively. The positive predictive value was 63.4% and the negative predictive value was 76.0%. Although the PRT test was specially designed for the assessment of tear volume, the accuracy was worse than the SP-CTT.

The Wv, defined by the summation of the NITBUT value and the SP-CTT or the
PRT values, were unable to give any useful information on the success of soft contact lens wear.

Seasonal change in HK does not appear to have a significant impact on the signs and symptoms of dry eye during soft contact lens wear. It is probably because the relative humidity in HK, when the study was conducted, was generally high. The result of the current study was therefore unlikely to be affected by seasonal change. Also, during the waking hour, while wearing their lenses, and when they were assessed, our subjects were working indoors, usually offices where the relative humidity was relatively constant. Hence, we did not really assess the change in humidity per se.

8.2 Implication of the results

When a cut-off point of 12.5 mm/15 s was used, the sensitivity of the SP-CTT was not as high as we hoped. It is therefore not adequate to use this test to confirm whether a potential soft lens wearer would be a suitable candidate or not. However, from the clinical point of view, before a patient is fitted with contact lenses, both the practitioner and the patient are concerned about the chance of wearing the contact lenses successfully. Here the positive and negative predictive values of the test could be useful. The positive and negative predictive values of the SP-CTT were 63.4% and 76.0% respectively, when the cut-off point was 12.5 mm/15 s. In other words, if a patient has a SP-CTT value <12.5 mm/15 s, s/he will have >60% chance of having dry eye problems during soft contact lens wear. On the contrary, if a patient has a SP-CTT value ≥12.5 mm/15 s, s/he will have >70% chance of being successful in wearing the soft contact lenses. This test can therefore be used as a screening test before patients are fitted with soft contact
lenses. Practitioners should communicate with their patients in order to assess their motivation and to tell them about the possibility of having signs and symptoms, so as to avoid unrealistic expectations. This can help to decrease the drop out rate due to dry eye problems leading to reduced wearing time. Possible treatments may also be discussed. Patients with stronger motivation may be able to comply with stricter wearing regimen such as reduced wearing time, extra careful lens care, rehydration of lenses at regular intervals during the day, more frequent aftercare consultations etc.

Our results showed that tear volume was the most important factor when determining the success of soft contact lens wear. This suggested the possibility of increasing the chance of successful soft contact lens wear by enhancing the bulk of the tear volume. Possible means includes the use of tear supplements, punctum occlusion, drinking more water, hormonal treatment…etc. but the details of these methods are beyond the scope of our discussion here.
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Appendix I

The McMonnies Dry Eye Questionnaire

A. Please answer the following by underlining the response most appropriate to you.

Age: Under 25 years 25 to 45 years Over 45 years

B. Currently wearing

No contact lenses Hard contact lenses Soft contact lenses

1. Do you ever experience any of the following eye symptoms? (Please underline those that apply to you)


2. How often do your eyes have these symptoms? (underline)

Never Sometimes Often Constantly

3. Have you ever had drops prescribed or other treatment for dry eyes?

Yes No Uncertain

4. Do you suffer from arthritis?

Yes No Uncertain

5. Do you suffer from thyroid abnormality?

Yes No Uncertain

6. Do you experience dryness of the nose, mouth, chest, or vagina?

Never Sometimes Often Constantly

7. Do you regard your eyes as being unusually sensitive to cigarette smoke, smog, air conditioning, central heating?

Yes No Sometimes
8. Do your eyes easily become very red and irritated when you are swimming in chlorinated fresh water?

Not applicable  Yes  No  Sometimes

9. Do you take (please underline) antihistamine tablets or use antihistamine eyedrops, diuretics (fluid tablets), sleeping tablets, tranquilizers, oral contraceptives, medication for duodenal ulcer or digestive problems or for high blood pressure?

or __________________________ (write)

10. Are your eyes dry and irritated the day after you have been drinking alcohol?

Not applicable  Yes  No  Sometimes

11. Are you known to sleep with your eyes partly open?

Yes  No  Sometimes

12. Do you have eye irritation as you wake from sleep?

Yes  No  Sometimes
Appendix II

Questions for study of effect of seasonal change on dry eye symptoms.

1. 閣下曾否感受以下任何眼部不適(請圈出適合的感覺)?
   a. 眼睛疼痛 b. 瘙 c. 眼乾 d. 磚物感 e. 熱辣感

2. 閣下的眼睛是否經常有以上症狀？(請圈出)
   a. 從來沒有 b. 間歇性 c. 時常 d. 一定有

English version of the above questions.

1. Do you ever experience any of the following eye symptoms? (Please circle those that apply to you)


2. How often do your eyes have these symptoms? (Please circle)

   Never Sometimes Often Constantly