Modification of the tear function index and its use in the diagnosis of Sjögren’s syndrome

Stephen B Kaye, Gillian Sims, Colin Willoughby, Anne E Field, Lesley Longman, Malcolm C Brown

Abstract

Background—The tear function index (TFI) has been shown to be of value in the diagnosis of patients suffering from Sjögren’s syndrome. It is dependent, however, on introducing into the conjunctival fornix the correct concentration of fluorescein in at least one and a half times the normal tear volume. The stimulus and effect of this added volume on the tear dynamics is likely to vary between individuals. These factors, together with the method of performing the test, limit its general applicability.

Aim—To devise a method of performing the TFI with less variability and more general applicability. To present a theoretical and in vitro assessment of the dynamics of the TFI.

Method—The study was divided into three parts. The first part was to compare the results obtained using a prepared strip containing 1.3 µl of 0.5% fluorescein with the introduction of the same amount of fluorescein as a drop. The second part was to compare the results obtained with prepared strips with the standard method of performing the TFI, both with and without topical anaesthetic. The third part was an in vitro study of the rate of flow of graded volumes on a filter paper strip. 42 subjects with a diagnosis of Sjögren’s syndrome according to the European criteria and 126 without Sjögren’s syndrome were included.

Results—There was no significant difference between the results obtained with a prepared strip and the introduction of 1.3 µl into the eye before performing the Schirmer’s test and TFI (0.1<p<0.93). There was, likewise, no significant difference between using the prepared strips and the standard method of performing the TFI (0.36<p<0.93). There was, however, less interocular difference (p=0.01) using the prepared strips than using a drop of fluorescein. Patients with Sjögren’s syndrome had mean TFIs of 11.7 and 8.61 with upper 95% confidence values of 15 and 12 without and with topical anaesthetic, respectively. The theoretical calculation of the TFI was similar to the observed values. The in vitro results allow the filter paper to be removed from the eye at any interval and to estimate the volume of tears that the filter paper was in contact with.

Conclusion—The proposed method of performing the TFI is easy to perform, reliable, and therefore has general applicability for primary care and general practitioners. It allows the rapid identification of subjects who may be suffering from Sjögren’s syndrome.

The Schirmer’s test is widely used in the diagnosis of dry eye syndrome. It has, however, a low sensitivity, specificity, and reproducibility.6–9 As one of the factors influencing the results of the Schirmer’s test is tear drainage, the tear clearance rate (TCR)7 was developed as a modified form of the fluorescein clearance test.5 The quotient of the Schirmer’s test and TCR, that is the tear function index (TFI) developed by Xu et al6 reflects both secretory and drainage conditions. The TFI was reported by Xu et al6 to provide better separation of normal, non-Sjögren’s syndrome and Sjögren’s syndrome dry eye patients, with better sensitivity and specificity than the Schirmer’s or TCR alone.

The TFI is performed by instilling fluorescein into the conjunctival fornix, followed by a Schirmer’s test. The colour (dilution) of the fluorescein on the filter paper strip is then compared with known standards to give the TCR; the TFI is then calculated by dividing the Schirmer’s value by the TCR. This method is, however, dependent on using a micropipette to instil the correct amount (10 µl) and concentration of fluorescein into each eye. It is also necessary for the Schirmer’s test to be performed at a specific time—that is, 5 minutes after instillation of fluorescein into the conjunctival fornix. Furthermore, instilling 10 µl of fluorescein (at least 1.5 times the normal tear volume) increases the tear volume and acts as a stimulant, so that the tear volume may not have returned to its initial value after 5 minutes, before commencement of the Schirmer’s test. This may then lead to an artificially high Schirmer’s test and TFI, particularly in patients who have abnormalities of tear drainage. These factors limit the general application of the TFI in practice. The purposes of this study were to devise a practical method for performing the TFI, and to present a model to describe the volume, distance, and time characteristics associated with the Schirmer’s test and TFI.

Study design

The first part of the study was to determine clinically whether the introduction of a drop of...
fluorescein into the conjunctival fornix produced the same TCR and TFI as using a filter paper strip with one end impregnated with the same amount of fluorescein. The standard technique of performing the TFI uses 10 µl of 0.5% fluorescein placed into the conjunctival fornix 5 minutes before performing the TFI. As this amount—that is, 10 µl of fluorescein, would wet the filter strip to approximately 20 mm, prepared strips containing this amount of fluorescein could not be used. In addition, although it is possible to approximate the amount and concentration of fluorescein remaining in the tear film after 5 minutes for given parameters (as discussed below), because tear film secretion, evaporation, and drainage vary between individuals, different amounts and concentrations of fluorescein would, therefore, be present after 5 minutes. Thus, because the volume of fluorescein remaining in the eye after 5 minutes is unknown, comparison of the TFI using a prepared strip or drop of fluorescein needed first to be assessed by introducing the same amount of fluorescein in the drop as in the prepared strip, immediately before performing the Schirmer’s test. The initial part of the study therefore, was to determine whether a prepared strip containing the same amount of fluorescein as a drop produced the same dynamics as reflected in the TCR and TFI. Providing this could be demonstrated, the second part of the study was to determine whether a prepared strip produced similar results to the standard method of performing and measuring the TFI. This would also allow the determination of confidence intervals for normal subjects and for those with an established diagnosis of Sjögren’s syndrome.

To determine a theoretical model for the TFI to assess the in vivo results, in vitro data were collected for the distance and rate of flow of graded volumes on strips of filter paper. Best fit curves were then used to characterise flow along the filter paper strip. A mathematical model was developed to determine the theoretical TFI and this was compared with the in vivo results.

Methods
Filter paper strips (Sno strips, Chauvin Pharmaceuticals Ltd) were used for the study. These strips are frequently used in the UK and are 5 mm in width for the first 15 mm and 6.5 mm in width for the remaining 25 mm. Filter paper strips were prepared by placing 1.3 µl of 0.5% fluorescein (Chauvin Pharmaceuticals Ltd) onto the end of the filter paper strip. This volume was chosen as it resulted in staining of the filter paper up to the notch of the strip (Fig 1). The prepared strips were then air dried and stored in a sterile universal container for not longer than 1 week until use.

PART ONE: FLUORESCIN FILTER PAPER STRIP COMPARED TO EQUIVALENT AMOUNT OF FLUORESCIN
The Schirmer’s test, TCR, and TFI were performed either by placing a prepared strip containing fluorescein or by placing 1.3 µl of 0.5% fluorescein into the conjunctival fornix, followed (approximately 5 seconds later) by the placement of a strip of filter paper over the junction of the middle and lateral third of the lower lid. The right eye always received the filter strip first, followed by the left eye within 5 seconds. The test was performed with the subject’s eyes closed. This was in order to reduce the variation produced by evaporation, which is dependent on the surface area and hence the size of the palpebral aperture, particularly for patients with Sjögren’s syndrome who may have increased or decreased evaporation rates. The effect of this is that patients with keratoconjunctivitis sicca (KCS) may lose the majority of their tear film through evaporation, compared with a 10% loss of the normal tear production.

After 3 minutes (part one) the filter paper was removed and the distance of the end of the wetted portion to the notch measured to the nearest millimetre. Three minutes was chosen for the first part of the study in order to minimise subject discomfort and because approximately 90% of wetting of the filter strip occurs in the first 3 minutes. The filter paper was then allowed to air dry and the colour of the filter paper, between the notch and wet mark, was compared with a standard dilution range. The standard dilution range was prepared using doubling dilutions of fluorescein starting at 0.5% and diluting to 1:128. A TCR of 1, therefore, reflects a concentration of fluorescein of 0.5%. The most comparable colour dilution was chosen by the observer and recorded as the TCR. Each strip of filter paper
Modification of the tear function index and its use in the diagnosis of Sjögren's syndrome

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99mTc)15 16 ; labial gland biopsy was undertaken
flow under standardised conditions14 and scin-
measurement of whole, unstimulated salivary
Salivary gland involvement was ascertained by
according to the diagnostic criteria proposed
A diagnosis of Sjögren's syndrome was made
SUBJECTS
any topical medication to either eye.
lateral external eye disease, if they had had any
filter paper into the eye. Five minutes later a filter strip was placed
pared filter strip into the fellow eye. Subjects
performing a Schirmer's test and placing a pre-
conjunctival sac of one eye 5 minutes before
Ten µl of 0.5% fluorescein were placed into the
tigraphy (radioisotope study of salivary gland
Although there is considerable variability in
the reproducibility of the within-eye Schirm-
er's test,1,6 the within-subject results for right
and left eyes are similar with no significant dif-
ference.1 It was decided, therefore, to com-
pare within-subject right and left eyes using a
prepared strip in one eye and a drop in the
other and vice versa, a prepared strip into both
eyes and a drop into both eyes, and then to
calculate the interocular difference for each test
procedure. The right eye received both the
drop and filter paper first, so that the delay
between receiving the drop and filter paper was
equivalent for right and left eyes.
Because of the exponential scale for the
tCR and TFI, the data were converted to
logarithmic form.9 The natural logarithm of
the right to left ratio for the TFI and TCR,
therefore, reflects the interocular difference.
Differences in means and within-eye and
within-group variances were then compared
using an F test.

PART TWO: STANDARD TFI VERSUS FLUORESCEIN
IMPREGNATED FILTER PAPER STRIP
Ten µl of 0.5% fluorescein were placed into the
conjunctival sac of one eye 5 minutes before
performing a Schirmer's test and placing a pre-
pared filter strip into the fellow eye. Subjects
were requested to keep their eyes closed, and
the filter strips were removed after 5 minutes.
Thirty to 60 minutes later, the procedure was
repeated, except that the 10 µl dilution of 0.5%
fluorescein was made using 0.4% oxybupro-
caine (Chauvin Pharmaceuticals) as the dilu-
ent and 10 µl of 0.4% oxybuprocaine was placed
into the conjunctival fornix of the fellow
eye. Five minutes later a filter strip was placed
into the eye receiving the 10 µl of fluorescein
and a prepared filter strip placed into the eye
that received 10 µl of oxybuprocaine. Subjects
were consecutively alternated for either the left
or right eye to receive the prepared strip. If the
right eye received the drop of fluorescein, then
the left eye would receive the drop of
fluorescein with oxybuprocaine. Filter paper
strips were then removed, air dried, and read as
in part one.
Patients were excluded if both parts of the
test—that is without and with topical anaes-
thetic, could not be completed, if they had uni-
lateral external eye disease, or if they were using
any topical medication to either eye.

SUBJECTS
A diagnosis of Sjögren's syndrome was made
according to the diagnostic criteria proposed
by the European Community Study Group.11
Salivary gland involvement was ascertained by
measurement of whole, unstimulated salivary
flow under standardised conditions16 and scin-
tigraphy (radioisotope study of salivary gland
function using technetium pertechnetate,
99mTc)15 16; labial gland biopsy was undertaken
by an oral physician using a standardised tech-
nique.10 Patients were included with definite
Sjögren's syndrome if they had four or more of
the European criteria11 for a diagnosis of
Sjögren's syndrome, including the presence of
autoantibodies.13
Patients were assessed for evidence of
meibomian disease using the features de-
scribed by Bron et al17 Shimazaki et al,18 and
Pfugfelder et al.19 Patients were included if
they were diagnosed as having obstructive mei-
boimian gland ductules (MGD): irregularity of
the lid margins, vascularity and retro place-
ment of mucocutaneous junction; involvement
of at least 30% of the meibomian glands with
metaplasia of the MGD, and obstruction of at
least three out of five glands using the method
of Pfugfelder et al.17 That is, digital compres-
sion of the lower lid just below the lash line and
the upper lid just above the lash line against the
lobe over an area spanning five visible meibo-
man gland orifices.20

PART THREE: IN VITRO DISTANCE AND RATE OF
FLOW OF GRADED VOLUMES ON FILTER PAPER
STRIPS
To determine the volume distance and volume-
time relation using a filter strip, the following
method was adopted. The filter paper strip was
folded at the notch, which is situated 5 mm
from the rounded end, and placed over the
edge of a thin glass plate, with the remainder
of the strip hanging vertically—similar to the
method of Clinch et al.2 A millimetre ruler was
situated alongside the filter paper. A drop of
fluorescein 2% was placed on the first,
horizontal part of the strip, and the maximum
distance that the front of fluorescein travelled
was measured to the nearest half millimetre.
The starting volume was 1.5 µl (1.3 µl was
found to wet the end of the strip up to the
notch at 5 mm) with 0.5 µl steps up to 15 µl.
The test was then repeated five times on three
separate occasions, in which the time taken for
the fluorescein front to reach each millimetre
up to the maximum distance (as determined
from the volume-distance measurements) was
recorded. Recording was done with a compu-
ter program with a continuous time display.

Results
PART ONE: FLUORESCEIN FILTER PAPER STRIP
COMPARSED WITH EQUIVALENT AMOUNT OF
FLUORESCEIN
In all, 168 subjects were included (mean age
66.3 years, SD 14.9), comprising 42 patients
with known definite Sjögren's syndrome, 32
patients with meibomian gland disease attend-
ing the external eye disease clinic, and 126
patients with a healthy ocular surface attending
for visual deterioration due to cataract forma-
tion or age related macular degeneration. Sub-
jects were consecutively divided into four
groups so that each group contained similar
proportions (1:1:3) of patients with Sjögren's
syndrome and or meibomian gland dysfunc-
tion and subjects with a healthy ocular surface.
Group 1: right eye: 1.3 µl 0.5% fluorescein;
left eye: prepared strip, 60 subjects
Table 1  Prepared strip containing 1.3 µl 0.5% fluorescein into left eye (group 1) or right eye (group 2), and 1.3 µl of 0.5% placed into inferior fornix of fellow eye. Schirmer’s test read after 3 minutes. A TCR of 1 reflects a concentration of fluorescein of 0.5% at 3 minutes. Log TFI is the natural logarithm

<table>
<thead>
<tr>
<th>Schirmer (mm)</th>
<th>TCR</th>
<th>Log TFI</th>
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<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Prepared strip into left eye and drop into right eye</td>
<td>Mean</td>
<td>15.81</td>
</tr>
<tr>
<td></td>
<td>(SD)</td>
<td>(10.1)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.93</td>
<td>0.18</td>
</tr>
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</table>

Table 2 1.3 µl of 0.5% fluorescein into both eyes (group 3). Schirmer’s test read after 3 minutes. A TCR of 1 reflects a concentration of fluorescein of 0.5% at 3 minutes. Log TFI is the natural logarithm

<table>
<thead>
<tr>
<th>Schirmer (mm)</th>
<th>TCR</th>
<th>Log TFI</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Mean</td>
<td>23.59</td>
<td>20.88</td>
</tr>
<tr>
<td>(SD)</td>
<td>(14.6)</td>
<td>(11.5)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.44</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 3 Prepared filter paper strips containing 1.3 µl of 0.5% fluorescein, placed into both eyes (group 4). Schirmer’s test read after 3 minutes. A TCR of 1 reflects a concentration of fluorescein of 0.5% at 3 minutes. Log TFI is the natural logarithm

<table>
<thead>
<tr>
<th>Schirmer (mm)</th>
<th>TCR</th>
<th>Log TFI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Mean</td>
<td>19.50</td>
<td>17.97</td>
</tr>
<tr>
<td>(SD)</td>
<td>(17.1)</td>
<td>(15.4)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.14</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Group 2: right eye: prepared strip; left eye: 1.3 µl 0.5% fluorescein, 40 subjects
Group 3: both eyes: 1.3 µl of 0.5% fluorescein, 30 subjects
Group 4: both eyes: prepared strip, 38 subjects.

Groups 1 and 2
There was no significant interocular difference in the Schirmer’s test, TCR, or TFI, whether a prepared strip or drop was used to perform the test (Tables 1–3). There was no significant interocular difference in the Schirmer’s, TCR, or TFI using a prepared strip in one eye and a drop in the other eye (Tables 1–3). Furthermore, there was no significant difference in the Schirmer’s, TCR, or TFI, whether a prepared strip or drop of fluorescein was placed in the right or left eye, respectively (Table 1) (groups 3 and 4). The interocular similarity was apparent over a wide range of values for the Schirmer’s, TCR, and TFI, that is, there was no correlation between the interocular difference in Schirmer’s test or TFI and the mean interocular value (p=0.47 and p=0.65). There was a significant correlation between right and left eyes using a prepared strip, drop or combination, for both the Schirmer’s test (r = 0.94, 0.81, 0.86, p<0.001) and the TFI (r = 0.94, 0.73, 0.57, p<0.001).

The variability of one observer in reading the Schirmer’s, TCR, and the resultant TFI, was assessed by comparing the within-eye variance of three independent readings using both the prepared strip and drop of fluorescein. This was done for the prepared strips into the right eye and drop into the left, and then vice versa. There was no significant difference in the within-eye variance of the Schirmer’s, TCR, or TFI whether the drop was placed into the right or left eye, respectively (p=0.39). The reliability, therefore, of measuring and reading the Schirmer’s test, TCR, and TFI was not dependent on whether a prepared strip or drop of fluorescein was used. There was a significant linear correlation between the Schirmer’s test and the TFI, for the prepared strips and drops (r=0.82 and 0.79, p=2×10⁻²⁰ and p=7×10⁻²²). There was no difference in the coefficient of regression whether a prepared strip or drop was used (Fisher’s z transformation on r; p=0.78).

Groups 3 and 4
On comparing a prepared strip with a drop into both eyes (Tables 2 and 3), there was no significant difference in the mean interocular difference for the Schirmer’s test using a drop (2.71, SD 8.54) or a prepared strip (1.82, SD 3.73) (p=0.09). The mean and variance of the interocular difference ratio for the TFI was, however, lower using a strip (1.08, SD 0.98) than using a drop (2.91, SD 4.29) (p=0.001 and p=0.01).

PART TWO. TFI: STANDARD METHOD VERSUS PREPARED FILTER PAPER STRIP
One hundred patients, 30 with a known definite diagnosis of Sjögren’s syndrome, 30 patients with meibomian gland disease, and 34 patients with a healthy ocular surface attending for visual deterioration due to cataract formation or age related macular degeneration were included. The average age for patients with Sjögren’s syndrome was 67.3 (SD 14.33, female: male 4.2:1) and those without Sjögren’s syndrome was 62.5 (SD 13.8, female: male 3:2.2).

Without and with topical anaesthesia
For both patients with and without Sjögren’s syndrome, there was no significant difference in the mean Schirmer’s test, TCR, or TFI for the prepared strip and drop, whether topical anaesthesia was or was not used (Tables 4 and 5). Six patients without Sjögren’s syndrome had Schirmer’s test values equal to or less than 5 mm. No subject without Sjögren’s syndrome, however, had a TFI of less than 40. There was no significant difference between subjects with MGD and those subjects with no external eye disease for the Schirmer’s test (p=0.79), TCR (p=0.66), or TFI (p=0.61).

Anaesthetic versus no anaesthetic
For the group of patients without Sjögren’s syndrome, there was a significant difference in the Schirmer’s test without and with anaesthetic, for the prepared strip (p=0.03) and drop (p=0.005), respectively. Likewise there was a significant difference in the TCR and TFI for the prepared strip (p=0.02, p=0.002) and drop (p= 0.005, p=0.001), respectively. For the patients with Sjögren’s syndrome, however, there was no significant difference in the Schirmer’s test (p=0.57 and p=0.78), TCR
Modification of the tear function index and its use in the diagnosis of Sjögren's syndrome

Table 4  Prepared strip (Pre-S) containing 1.3 µl of 0.5% fluorescein placed into the inferior fornix 5 minutes after instilling 10 µl of 0.5% fluorescein into inferior fornix of the fellow eye. Schirmer's test read after 5 minutes. A TCR of 1 reflects a concentration of fluorescein of 0.5% at 5 minutes. Log TFI is the natural logarithm

<table>
<thead>
<tr>
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<th>TCR</th>
<th>TFI</th>
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<tbody>
<tr>
<td></td>
<td>Pre-S</td>
<td>Drop</td>
</tr>
<tr>
<td>Non-Sjögren's syndrome</td>
<td>13.7</td>
<td>14.7</td>
</tr>
<tr>
<td>SD</td>
<td>6.36</td>
<td>7.14</td>
</tr>
<tr>
<td>p Value</td>
<td>0.36</td>
<td>0.73</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>2.53</td>
<td>2.66</td>
</tr>
<tr>
<td>SD</td>
<td>1.55</td>
<td>1.84</td>
</tr>
<tr>
<td>p Value</td>
<td>0.37</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Table 5  Prepared strip (Pre-S) containing 1.3 µl of 0.5% fluorescein placed into inferior fornix 5 minutes after instilling 10 µl of 0.4% benoxinate into the same eye and 5 minutes after instilling 10 µl of 0.5% fluorescein diluted in 0.4% benoxinate into the fellow eye. Schirmer's test read after 5 minutes. A TCR of 1 reflects a concentration of fluorescein of 0.5% at 5 minutes. Log TFI is the natural logarithm

<table>
<thead>
<tr>
<th></th>
<th>TCR</th>
<th>TFI</th>
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<tbody>
<tr>
<td></td>
<td>Pre-S</td>
<td>Drop</td>
</tr>
<tr>
<td>Non-Sjögren's syndrome</td>
<td>11.5</td>
<td>11.5</td>
</tr>
<tr>
<td>SD</td>
<td>4.68</td>
<td>5.10</td>
</tr>
<tr>
<td>p Value</td>
<td>0.52</td>
<td>0.618</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>2.29</td>
<td>2.54</td>
</tr>
<tr>
<td>SD</td>
<td>1.66</td>
<td>1.34</td>
</tr>
<tr>
<td>p Value</td>
<td>0.27</td>
<td>0.152</td>
</tr>
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(p=0.02 and p= 0.26), or TFI (p=0.20 and p=0.60) for both the prepared strip and drop, whether topical anaesthesia was or was not used.

PART THREE: IN VITRO RESULTS

As the filter paper used was 5 mm in diameter for the first 15 mm and 6.5 mm in diameter for the remainder of the strip, the measurements were separated into two groups, 5–15 mm and greater than 15 mm.

Volume-distance relation

The relation between volume and distance travelled on the filter paper, was best approximated by the quadratic function $D = 2.05 + 2.38 V - 0.042 V^2$ ($r=0.998$, $p < 0.001$) for volumes 0–15 µl (0–28.5 mm), using a computer generated least squares approximation (spss version 9) to the data. We found a greater distance travelled for a given volume than that of fluorescein itself moves at a different rate from other water or an electrolyte concentration as is found in tears.

Volume-distance-time

The time taken for a given volume to reach the maximum distance along the filter paper could be approximated by the power function, time $= 2.2 + V^{1.3}$ ($r=0.9$ $p<0.001$), using a computer generated least squares approximation (spss) to the data. The time taken for a given volume to move a specified distance also followed a power function ($r=0.92$ $p<0.001$ for the distances 5–15 in 1 mm steps) and is shown in Figure 2. In essence, larger volumes moved more quickly than smaller volumes. As shown in Figure 2, a volume of 5 µl took 7 seconds to travel 10 mm, while a volume of 10 µl took 3.5 seconds to travel 10 mm. Extrapolating to the in vivo situation, removing the filter paper from the eye after any length of time, and measuring the distance travelled, allows one to determine the volume of tears that was in contact with the filter strip. For example, if the filter strips are removed after 1 minute, and the distances travelled as measured from the notch (5 mm from the end) are 15 mm and 13 mm, this reflects a volume of tears of at least 10 µl and 8 µl, respectively. Alternatively, if the filter paper strips are removed after $1/2$ minute and the distances travelled, as measured from the notch, are 10 mm and 8 mm, this reflects a volume of tears of at least 7 µl and 5 µl, respectively.

Theoretical TFI

If one considers the starting volume of tears as $V_0$, the production of tears or rate of inflow as $I$, the rate of loss of tears due to evaporation as $P$, the rate of loss of tears due to outflow as $E$, then the net rate of inflow ($I-N$) is $I-P$, and the net rate of flow (N) either into or out of the eye is, therefore, $I-N-E$. If a substance such as fluorescein is added to the tears, and assuming immediate and perfect mixing, then loss of fluorescein only occurs from outflow—that is, E. Although there is likely to be some compartmentalisation the following analysis is generally applicable. The rate of change in concentration of a substance added to the tears is proportional to the concentration of the substance and inflow and inversely proportional to the sum of the volume and net flow—that is,

$$\frac{dC(t)}{dt} = -C(t)IN/(V_0+Nt) \quad (1)$$

If the volume of tears remains constant, equal to the initial volume ($V_0$)—that is $I = I_0$, so that $N = 0$ then solving equation (1), the concentration of fluorescein at a time $t$, can be calculated from

$$C(t) = C(0) e^{-t/V_0} \quad (2)$$
where $C(0)$ is the starting concentration—that is, when $t = 0$. For example, if the volume of tears is approximately 7 µl, and the net inflow equal to outflow is 1 µl, the concentration after 5 minutes would reduce to $C(5) = 0.49 C(0)$.

If, however, the net inflow $I_n$ and outflow $E$ differ—that is, $N \neq 0$, then the volume of tears will change, so that the volume at some time $t$ will be $V(t) = V_0 + Nt$. Solving equation (1), where $N$ is not zero, leads to

$$\ln C(t) + \ln(1 + Nt/V_0) = K$$

where $K$ is a constant. When $t = 0$, the starting concentration $C(0)$ is

$$\ln C(0) + \ln(1 + N/V_0) = K$$

Substituting equation (4) into equation (3), $N \neq 0$, the concentration $C(t)$ of fluorescein at time $(t)$ follows the relation

$$C(t) = C(0)/(1 + Nt/V_0)$$

In this scenario where, say, $I_n > E$ due to, say, reflex epiphora, then if $I_n$ increases to 4 µl/min for 5 minutes, and $E$ remains at 1 µl/min, the concentration after 5 minutes, assuming a starting volume of 7 µl, would be $C(5) = 0.22 C(0)$.

Although it is unclear what effect 10 µl of fluorescein instilled 5 minutes before performing the TFI would have on the tear volume and concentration, a prediction based on the above models can be made. The addition of a 10 µl drop of fluorescein would increase the volume to 17 µl, with a starting fluorescein concentration $C(0)$ of $10/17 \times 0.5\%$ (0.5% fluorescein, represents a TCR of 1). If, after 5 minutes, one makes the assumption that the tear volume has returned to 7 µl, then the outflow over this 5 minute period would have been 15 µl or 3 µl/min (inflow 1 µl/min over this period). The difference ($N$) between inflow (1 µl) and outflow over this 5 minute period would then be $-2$ µl/min, so that the change in concentration of fluorescein after the first 5 minutes would be $C(5) = 0.38$ (or 0.64 for 2 µl starting tear volume and a net inflow of 0.3 µl/min and outflow of 2.3 µl/min). The concentration of fluorescein after the second 5 minutes when the TFI is measured would then be $C(10) = 0.38 \times e^{-2 \times 5} = 0.18$ (0.30 for 2 µl starting tear volume). Thus, the expected TCR would be 0.18, giving an expected TFI (assuming a Schirmer’s value of 12 mm) of 67, or 10 for a patient with Sjögren’s syndrome, assuming 3 mm for the Schirmer’s test (equivalent to a volume of 2 µl). Patients with KCS have been reported to have $I$ of <0.5 µl/min. An I of 0.5 corresponds (from Mishima et al\cite{23} and Jordan and Baum\cite{24}) to a volume of approximately 2 µl.

Using a prepared strip containing 6.5 µg of fluorescein (1.3 µl × 0.5%), the starting concentration would be 6.5 µg/7 µl, which is equivalent to a TCR of 6.5/35, where 0.5% fluorescein (5 µg/µl) represents a TCR of 1.

The TCR after 5 minutes would be $C(5) = 6.5/35 e^{-1 \times 5} = 0.09$, for a starting volume of 7 µl giving a TFI of 13.9. For a patient with Sjögren’s syndrome the TCR for a tear volume of 2 µl would be 31.3 and assuming a Schirmer’s test value of 3 mm, the TFI would be 9.7. These values—that is, 9.5–10 for a subject with Sjögren’s syndrome, are similar to the observed values, while those expected for a subject without Sjögren’s syndrome—that is, 67–133, are less than found. This very likely reflects the reflex secretion from the fluorescein and filter paper present in healthy subjects but usually absent in subjects with Sjögren’s syndrome.\cite{25,26}

For example, if stimulation from the filter strip increases the inflow to 2 µl/min, the TCR would reduce to 0.058 using a drop and 0.045 using a strip, which translates to a TFI of $12/0.058 = 207$ and $12/0.045 = 267$, respectively. This is also suggested if the values obtained by Mathers et al\cite{27} for tear volumes and Schirmer’s tests are considered. In the age group 61–70 years, they found a much lower normal tear volume of 1.73 µl with an inflow of 0.12 µl/min, much less than has previously been reported. Using these values, the change in concentration would be 0.35 giving a predicted TFI of 34 using a Schirmer’s value of 12 mm reported by Mather et al\cite{27} for this age range, which is much less than found in this study or in that by Xu et al.\cite{27} A Schirmer’s value of 12 mm corresponds to a tear volume in contact with the filter paper of at least 8 µl which, taken together with the TFI found in this study and that of Xu et al\cite{27}, indicates a substantial reflex component in non-Sjögren’s subjects.

**Discussion**

The Schirmer’s test remains the most popular test for the diagnosis of dry eye syndrome. Its poor sensitivity, specificity, and reproducibility\cite{28–30} often leads to equivocal results. Many other tests have therefore been introduced for the diagnosis of dry eye, such as tear break up time,\cite{31} lactoferrin,\cite{32} immunological assay, vital staining of the ocular surface,\cite{33} tear meniscus,\cite{34} height or tear osmolarity,\cite{35} among others. These tests reflect different aspects of the tear film, each providing different qualitative information. The Schirmer’s test, however, is easy to perform and, without the need for any additional equipment, has generalised applicability. Some of the variables associated with the Schirmer’s test have been overcome by the introduction of the TFI.\cite{26} A component of the TFI—that is the TCR, gives an indication of the dilution of fluorescein (on a doubling scale) that has occurred in the tear film. Using the TCR as the denominator in the calculation of the TFI, results in a 2$^n$ amplification of the Schirmer’s test value. This may account for the improved sensitivity and specificity of the TFI for the diagnosis of dry eye, and for distinguishing between Sjögren’s and non-Sjögren’s dry eye syndromes.\cite{26} A micropipette, however, is needed to instil the correct amount and concentration of fluorescein into each eye and it is necessary for the Schirmer’s test to be performed at a specific time after instillation of fluorescein into the conjunctival fornix. These practical factors limit the general application of the TFI in practice.

The first part of this study shows that using a prepared strip is equivalent in terms of the Schirmer’s test, TCR, and TFI to placing the equivalent amount of fluorescein in the con-
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V and ect on tear production. The assumption particularly over the 5 minutes before measurement of the TFI, depending on drainage, evaporation, and effect on tear production. The assumption that the tear volume has returned to its resting volume at the commencement of the test is likely to be inaccurate. Thus although there appeared to be no apparent difference in the Schirmer’s test, TCR, or TFI between the standard and modified method, the significant decrease in interocular variability using the prepared strip, is likely to reflect the variation in drainage which occurs with the standard method.

The method proposed for performing the TFI, does not require additional equipment to instil the correct amount and concentration of fluorescein into the eye, and is not dependent on performing the Schirmer’s test after a specific time. The TFI upper 95% confidence interval using prepared strips for the diagnosis of Sjögren’s syndrome, was 15 without and 12 with anaesthetic—an approximate Schirmer’s test value of 3 and a TCR of 0.25 or 1:4. The TFI was found to more reliably distinguish between patients with Sjögren’s syndrome and those without. That is, there were six subjects without Sjögren’s syndrome who had Schirm er’s test values equal to or less than 5 mm. Further studies will determine if the proposed confidence levels need to be adjusted for age and sex—particularly for an early age of onset of Sjögren’s syndrome.

The use of prepared strips thus provide a simple reliable method for performing the TCR and TFI, and is well suited to the general practitioner or primary care department. It allows both ophthalmologists and non-ophthalmologists to identify patients who may be suffering from Sjögren’s syndrome. Further investigation of such patients can then proceed along established criteria for the diagnosis of Sjögren’s syndrome.

The in vitro test data provides useful information, particularly for those situations, where the patient is unable to tolerate the filter strip for 5 minutes. It also allows the observer to remove the filter strip at any time interval and to estimate the approximate volume of tears that the filter paper was in contact with.

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Modification of the tear function index and its use in the diagnosis of Sjögren's syndrome

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