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The potential influence of Schirmer strip variables on dry eye disease characterisation, and on tear collection and analysis

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ABSTRACTS

Purpose: The use of the Schirmer strips (SS) as a tool in the characterisation of dry eye disease, depends upon the quantitative assessment of tear production and constituents. The aim of this study was to ascertain the extent to which the properties of commercially available SS can vary and the way in which this baseline information may relate to their comparability in clinical use.

Methods: Five SS were analysed: Clement Clarke®, TearFlo®, Bio Schirmer®, Omni Schirmer® and JingMing®. Various aspects of their physical appearance and physicochemical behaviour were measured, including size, weight, and thickness together with surface morphology (assessed by SEM) and aqueous uptake and release behaviour (including the influence of each strip on protein retention and eluent osmolarity).

Results: All physical parameters varied between the strips studied for example the Clement Clark was the largest, thickest, and heaviest strip assessed in this study. SEM images showed that each of the SS had unique surface morphologies. Statistically significant differences among the strips were found for uptake ($p = 0.001$) and release volume ($p = 0.014$). Clement Clarke absorbed the highest volume over a fixed time period (23.8 ± 1.6 µl) and Omni the lowest (19.3 ± 0.5 µl). Clement Clarke showing the highest eluent osmolarity value (5.0 ± 0.0 mOsm/L) and TearFlo the lowest (2.8 ± 0.4 mOsm/L).

Conclusion: The five strips investigated in this study indicate that there is no standardisation of commercial strips, despite the fact that the need for standardisation was recognised over fifty years ago. This study provides useful baseline information relating to SS comparability in clinical use.

1. Introduction

It is estimated that between 5 and 30% of the population suffer from dry eye disease (DED) and symptoms of dryness are very commonly reported by patients in eye care clinics [1–3]. Aqueous tear deficiency, which is related to a reduction of the lacrimal tear secretion and dysfunction, is one of the two main categories of DED and Schirmer strips (SS) are still widely used today to measure tear production for DED diagnosis [4–7]. The test was first described in 1903 by Otto Schirmer [8]. It uses absorbent filter paper strips, which are inserted into the temporal lower conjunctival sac and after 5 min the length of wetting of the strip is recorded in millimetres. It is generally accepted that a SS tear production reading of greater than 10 mm/5 min is accepted as the norm, and a reading below 5 mm/5 mins is indicative of tear deficiency and dry eye (DE), however neither of these values are absolute [9,10]. A more recent application of the Schirmer strips has been to collect tear samples for analysis of ocular biomarkers, the advantage of this approach being the fact that the device is well-established in clinical ophthalmic practice [11–13]. Accurate quantitation of tear components in tear fluid is not only important in understanding the physiological properties of tears, but also affords valuable diagnostic opportunities for the clinician [14]. It is only by recognition of the sources of error and in particular the variability of Schirmer strips in clinical practice, however, that the well-recognised problem of Schirmer reproducibility can be understood and minimised [15,16].

Standardisation of procedure has an important influence on the results obtained with the Schirmer test [17–20]. Differences are caused, for example, by variations in the eye gaze position, with higher results

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obtained when the Schirmer test is performed with an inferior gaze [17]. Similarly, differences arise when the test is performed with open, in contrast to closed eyes; closed eyes result in lower values, but these are likely to be more reliable, as eyelid margin effects, eyelash stimulation and local environmental conditions can alter the tear turnover rate [18,19]. It is equally important to recognise and quantify the effects of variations in the Schirmer strip material on the results obtained. Initially the Schirmer test used blotting paper which was cut into strips measuring 35 mm by 5 mm. Subsequently, litmus paper, cigarette paper and a number of other blotting papers were investigated [21–23]. Two standardised materials for fabrication of Schirmer strips have been proposed: Whatman standard No. 41 filter paper in 1953 [6] and Black Ribbon No. 589 in 1961 [24]. Although Whatman standard No. 41 or Black Ribbon No. 589 are still widely used in Schirmer strip fabrication today, the majority of manufacturers do not declare the origin or source of their strips.

There are, currently, many commercially available Schirmer strips, and even a simple visual inspection indicates that there are differences between them. The aim of this study was to collate comparative data on the relative behaviour of commercially available Schirmer strips using a variety of characterisation techniques relevant to the assessment of tear volume and the analysis of tear components. Dissimilarities between the strips have the potential to influence tear production measurements and affect diagnostic assessments. They may also affect the retrieved volume and perceived constituents of sampled tears.

2. Materials and methods

A representative sample of five commercially available SS was selected and assessed. (Table 1). They including one fluorescein incorporated strip, one without ruler markings and a spread of geographically sourced strips. Lot-to-lot variation was assessed and the standard deviation was found to be within the limits of the intra-lot variation for all the parameters investigated.

2.1. Physical parameters

The physical characteristics: appearance, size, thickness and weight of the five different SS were measured. Precise length was measured using a jeweller's eye piece with a 0.001 cm sensitivity. A microbalance with a microgram sensitivity range was used to measure weight, and a micrometer with an accuracy of 0.001 cm was used to measure thickness. Five individual strips of each type were measured.

Table 1
Selected Schirmer strips.

Schirmer strip	Manufacturer	Box details	Physical appearance
Clement Clarke	Haag-Streit Clement Clarke Intl. (UK)	50 pouches, each with 2 strips	
TearFlo	HUB Pharmaceuticals, LLC (USA)	100 individually packed strips	
Bio Schirmer	Biotech Vision Care (India)	100 individually packed strips	
Omni Schirmer	Omni Lens PVT. Ltd (India)	100 individually packed strips	
JingMing	Tianjin JingMing New Technological Development Co. Ltd (China)	50 pouches, each with 2 strips	

2.2. Uptake and release: volume

To measure the uptake volume, an 80 µl aliquot of phosphate buffed saline (PBS) was added to a round bottomed 2 ml microcentrifuge tube. The tip of each strip was dipped into the PBS reservoir for 1 min which enabled the capillary action of the strips, which is responsible for the in-eye wicking action used to 'fill' the strip, to be assessed. The wetted strip was then placed into a smaller 0.5 ml centrifuge tube in which a hole had been made at the base using a 0.6 mm gauge microlance. A microcentrifuge tube piggyback centrifugal set-up was then used. This was done by placing the 0.5 ml centrifuge tube into a larger 1.5 ml centrifuge tube and both were then centrifuged at 10,000 rpm for 5 min. The volume remaining in the 2 ml microcentrifuge tube was measured with a micropipette having a volume accuracy of 0.1 µl. (Uptake volume = starting volume (i.e. 80 µl) – remaining volume). The wetting length in millimetres was also recorded where applicable. The volume released was collected in the 1.5 ml centrifuge tube and measured immediately, also using the micropipette.

2.3. Osmolarity

Five strips of each type were immersed in 1 ml of deionised (DI) ultrapure water (Purite: resistivity 18.2 MΩ cm) or PBS and placed on a shaker at room temperature for 24 h. After the 24 h soak the strips were placed individually into a 0.5 ml microcentrifuge tube; the microcentrifuge tube centrifugal piggyback set-up (Sec 2.2) was again used. 100 µl of the resultant eluate was collected for osmolarity measurement. Each sample was measured on the automatic micro-digital osmometer (Type 6, CamLab, Cambridge, UK). Six measurements were performed with both DI and PBS separately. The osmometer was calibrated using known standards solutions (DI = 000 mOsm/L; PBS ≈285 mOsm/L). As a control, 1 ml of calibrant (DI or PBS) was aliquoted to an individual vial in the absence of a SS.

2.4. Uptake and release: protein concentration

For simplicity in these initial in vitro studies stages, a single protein species was chosen to investigate the potential interaction between the strip and tear proteins. Human albumin, which is upregulated in tears on SS insertion [25], was the obvious choice. It is a negatively charged protein with a molecular weight in the region of 66 kDa. An 80 µl aliquot of 1 mg/ml of human serum albumin was added to 2 ml microcentrifuge tube. The tip of each strip was dipped into the albumin solution reservoir for 1 min to mimic the in-eye wicking and capillary action used to 'fill' the strip. The individual strips were then placed into a 0.5 ml microcentrifuge and the microcentrifuge tube piggyback centrifugal set-up was again used. The resultant eluate was collected for total protein concentration measurements.

The volume remaining in the original 2 ml microcentrifuge tube and the volume released were measured with a micropipette. These volumes were used to calculate the actual microgram weight of the protein (as opposed to mg/ml concentration which would be volume dependent). Presenting the results in terms of weight negated volume dissimilarities between strips. Protein levels in the blank strip were also measured as a control by extracting the SS separately with deionised water and PBS.

Total protein concentration was measured using a microBCA Protein Assay Kit (Thermo Scientific, Rockford, USA), in accordance with the kit instructions. Briefly, 150 µl of standard/sample and 150 µl of working reagent were added to each designated well, of a 96 well plate. The plates were covered and incubated at 37 °C for 2 h. Absorbance at 562 nm was measured with a UV–vis spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale CA, USA). All analytes were measured in duplicate. Sensitivity limits are quoted at 2 µg/ml.