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Reproducibility and repeatability of the OcuSense TearLab™ osmometer

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Abstract
Background Some studies report that increased tear osmolarity is a reliable indicator of dry eye syndrome (DES). The OcuSense TearLab™ osmometer requires less than a 100-nl sample of tears and provides an instant quantitative result. Our aim was to clinically evaluate this instrument in terms of its reproducibility and repeatability.
Methods Twenty-nine participants who ranged in age from 19 to 49 years (mean±SD: 23.3±5.5 years) were recruited. Osmolarity readings were collected by two operators, in two sessions separated by 1 or 2 weeks in order to assess test reproducibility and repeatability.
Results The coefficient of reproducibility was 39 mOsms/l; the coefficient of repeatability was 33 mOsms/l.
Conclusions Our mean coefficient of variation over four readings for 29 subjects is 2.9%, which compares well with that reported by the manufacturer. Our results inform practitioners about the level of change over time that can be considered clinically relevant for healthy subjects. This value is 33mOsms/l; any change smaller than this could be attributed to measurement noise.

Keywords Dry eye · Osmolarity · Osmometer · TearLab · Tears

Introduction

Many techniques are available for use in the investigation of dry eye syndrome (DES), but they are not always reliable, and often more than one test is required to confirm a diagnosis [1]. Some studies have found that increased tear osmolarity is a reliable indicator of DES, and some investigators consider it to be a potential gold standard for diagnosis [1, 2]. Osmolarity is the total concentration of dissolved particles in a solution, irrespective of density, size, molecular weight, or electrical charges [1]. Tear osmolarity captures the balance of inputs and outputs from the tear film dynamics, and is the primary driver for normal homoeostasis, which regulates tear flow [2]. Evaporation of tears, a decrease in the production of tears, and meibomian gland dysfunction all cause an increase in tear osmolarity, such that measurement of tear osmolarity is suggested to be highly diagnostic for all types of DES [1].

Until recently, laboratory analysis was required to determine tear osmolarity, and this made investigation costly and time-consuming [1]. Tear osmolarity can be assessed using cryoscopy where a tear sample is frozen, and the time taken to thaw is used as an indirect measure of osmolarity. This is also known as the freezing point depression method. Although only 0.2 μl of tears are required (collected using a plastic capillary tube), they then have to be transferred into a 'Tear-Tip'. This transfer may introduce errors due to evaporation. Another limitation of this technique is that in some methods, such as when using the Clifton Osmometer, the observer is required to make a subjective judgment as to when melting has occurred [3].

A vapor pressure osmometer can also be used to measure tear osmolarity. This instrument makes use of the dew point depression, that is, the difference between the temperature that condenses the tears and the temperature that returns them to water vapor [4]. The technique is quick and compares well with the Clifton osmometer. However, vapor pressure osmometry requires saturating a cellulose acetate disc to collect the tears, and sometimes there are insufficient

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tears available on the ocular surface to achieve this [5]. Early versions of this osmometer required up to 5 μl of tears while a more advanced model only required 2 μl, this amount can still be difficult to collect from people with DES [4].

More recent techniques involve the use of electrical impedance to measure tear osmolarity and require only nanoliter samples of tears [6]. The OcuSense TearLab™ osmometer (OcuSense Inc., San Diego, CA, USA) is reported to require a tear sample of less than 100 nl [1, 7]. It uses a temperature-corrected impedance measurement of tear fluid to provide an indirect assessment of tear osmolarity [8] and provides an instant quantitative result [1, 7]. The system is portable, with a small base and two hand-held pens called tonopens, which are used to take the measurement. Each has its own docking space so that each eye can be tested consecutively without having to wait for the first test to be completed. The OcuSense TearLab™ osmometer uses the disposable Osmolarity Test Card (a single-use, non-sterile, polycarbonate microchip) for tear sample collection and analysis. This means that the risk of evaporation during transfer is eliminated. The chip contains a microfluidic channel to collect the tears and a gold electrode embedded in the polycarbonate card to enable measurement of the impedance of the sample [9]. This numeric value is subsequently displayed on the base of the instrument.

Our aim was to determine, for healthy subjects, the inter-session (repeatability) and inter-practitioner (reproducibility) measurement variability of the OcuSense TearLab™ osmometer. This is often referred to as measurement noise. Repeatability and reproducibility values give a sense of the magnitude required for a clinically relevant change in tear osmolarity. This is of particular interest since the OcuSense TearLab™ osmometer might be used to monitor the effect of interventions on osmolarity over time. As far as we are aware, this is the first independent clinical evaluation of this instrument.

Materials and methods

Subjects

Twenty-nine participants were recruited from the staff, students, and patients of Aston University Optometry Department. Ethical approval was obtained from the University's Office of Human Research. Written consent was obtained after each participant had been fully informed of the nature of the study according to the code of ethics in the Declaration of Helsinki protocol. None had been diagnosed with any ocular disease, or were taking prescribed medication, or using any ocular topical agents.

Procedure

Tear osmolarity was measured using the OcuSense TearLab™ osmometer. This osmometer is calibrated by the manufacturer [9]. However, the device is supplied with electronic check cards and these were used to reconfirm calibration at the start of each test session. Tonopens were used to take osmolarity readings from the tear meniscus on the lower eyelid. The lower eyelid was kept in its natural position and not pulled down.

Subjects were asked to attend for tear osmolarity testing with the OcuSense TearLab™ osmometer on two different occasions. They were asked not to swim or take part in vigorous exercise during the hours prior to their visit. Prior to tear osmolarity testing, the participants were asked whether their eyes felt particularly sensitive that day, and all responses were negative. All data were collected during the months of December and January, so that seasonal allergies such as hay fever were unlikely to confound the results. The two visits were arranged at the same time of day, on the same day of the week, but were separated by 1 or 2 weeks. It is important to make repeat measures at the same time of day as tear osmolarity has been reported to be low in mid-morning, high following lunch and throughout the afternoon, and then to decline again in the evening [10].

At each of the two visits, two tear osmolarity readings were taken. The measurements were always taken from the right eye as large differences have been reported between right and left eyes in some patients [11]. At the first visit, one of the authors (MA) always took the first tear osmolarity reading while another investigator (IA) always took the second; this order was reversed at the second visit. Only one reading was taken from each subject by each observer at each visit; the OcuSense TearLab™ osmometer is designed to give accurate tear osmolarity measurements in just a single reading. This observational study was conducted between January and July 2010.

Analysis of inter-practitioner reproducibility and inter-session repeatability involved calculating the mean difference in the tear osmolarity between data sets for each comparison. The degree of repeatability/reproducibility is the range over which 95% of the differences lie, i.e., the 95% limits of repeatability/reproducibility are equal to the mean difference ±1.96 multiplied by the standard deviation of the differences [12].

Results

We measured tear osmolarity for 29 subjects, 20 females and nine males, aged between 19 and 49 years (mean±SD: 23.3±5.5 years). The overall mean tear osmolarity values at the first visit were 329.2±16.0 mOsms/l (MA) and 326.6±13.1 mOsms/l (IA), and for the second visit were 328.5±14.2

Table 1 Coefficients of repeatability and reproducibility for tear osmolarity measured using the OcuSense TearLab™ osmometer. MA are the initials of investigator 1 and IA are the initials of investigator 2

	Repeatability		Reproducibility	
	MA1-MA2	IA1-IA2	MA1-IA1	IA2-MA2
Mean difference	1	–1	3	–1
Standard deviation of mean differences	16	17	20	12
Coefficient of repeatability/reproducibility	31	33	39	24

mOsms/l (MA) and 327.5±14.0 mOsms/l (IA). There was no correlation between the mean of the four tear osmolarity readings (328.0±9.5 mOsms/l) and age (r=–0.032, p=0.870).

The mean of the four tear osmolarity values was 329.1±9.6 mOsms/l for females and 325.6±9.2 mOsms/l for males, and these values were not significantly different (t=0.922, p=0.365). The coefficients of repeatability and reproducibility are shown in Table 1.

The comparisons resulting in the largest coefficients of reproducibility (MA1-IA1) and repeatability (IA1-IA2) have been plotted as difference against the mean plots in Figs. 1 and 2, respectively.

Discussion

We have measured tear osmolarity on 29 subjects using the OcuSense TearLab™ osmometer. The coefficient of repeatability values indicate the amount of change that can occur between readings and still be classed as measurement noise. Knowledge of an instrument's measurement variability (noise) is an essential aspect of separating normal from abnormal states. It is also important in the detection of significant change in the longitudinal assessment of any clinical condition. Test–retest reliability is a measure of a test's precision, rather than its accuracy, since the latter entails how closely matched the measurements are to their true value [13].

The coefficient of reproducibility values indicate the amount of change that can occur during the same data collection session between operators, and still be classed as measurement noise. Our data suggest that when different operators are taking OcuSense TearLab™ osmometer tear osmolarity readings during the same session, only increases or decreases of more than 39 mOsms/l can be classed as clinically relevant (see Fig. 1).

Our data suggests that when the same operator is taking repeated OcuSense TearLab™ osmometer tear osmolarity readings over time, only increases or decreases of more than 33 mOsms/l can be classed as clinically relevant (see Fig. 2). Considering our overall mean tear osmolarity value of 328±9.5 mOsms/l, the coefficient of repeatability represents 10% of the mean, and the coefficient of reproducibility represents 12% of the mean. This suggests that measurement noise is low.

The OcuSense TearLab™ osmometer manufacturers report that the instrument compares well with the Wescor Model 5520 vapor pressure osmometer calibrated to National

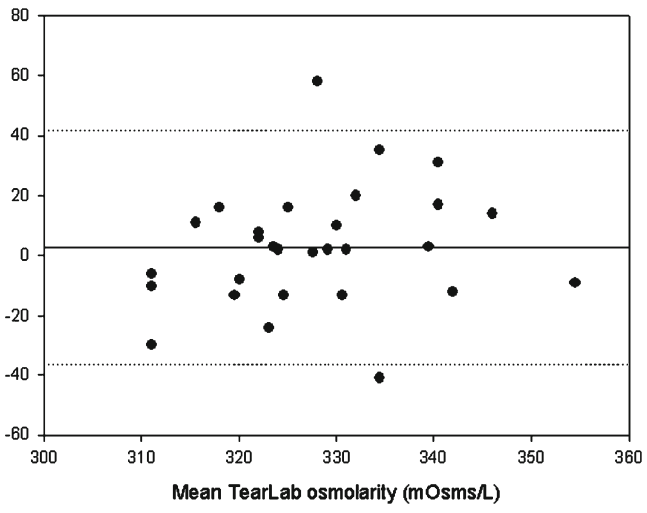

Fig. 1 Difference in TearLab tear osmolarity reading between MA1 and IA1, compared to the mean (n=29). The mean bias is represented by the *solid line*, and the 95% confidence limits are represented by the *dashed lines*. MA are the initials of investigator 1 and IA are the initials of investigator 2

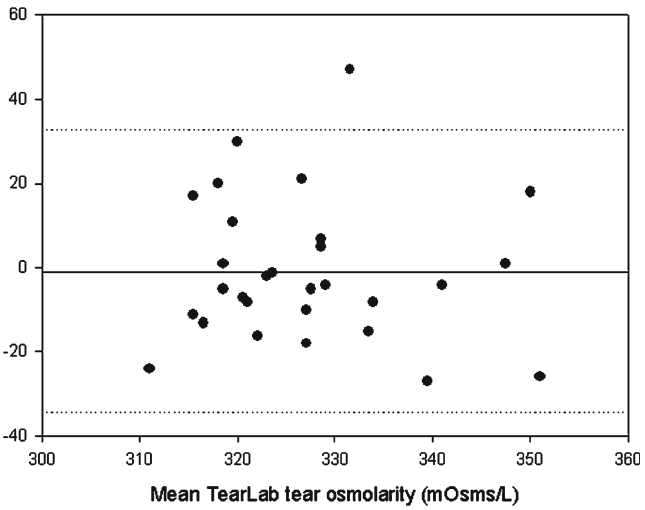

Fig. 2 Difference in TearLab tear osmolarity reading between IA1 and IA2, compared to the mean (n=29). The mean bias is represented by the *solid line*, and the 95% confidence limits are represented by the *dashed lines*

Institute of Standards and Technology (NIST) traceable standards, with a correlation coefficient of 0.98 from a sample of 28 [9]. They also state that the instrument has a coefficient of variation of approximately 1.5% [9]. Another group who used the OcuSense TearLab™ osmometer to assess dry eye treatment effectiveness report a coefficient of variation similar to this [7]. Our mean coefficient of variation over four readings for 29 subjects is 2.9%, and compares well with that reported by the manufacturer.

Our paper reports coefficients of repeatability and reproducibility for the OcuSense TearLab™ osmometer, which informs practitioners about the level of change over time and between practitioners that can be considered relevant. In other words, it informs practitioners about measurement noise within readings, and how large a change or difference in readings needs to be in order to be considered clinically relevant. Benelli and colleagues [7] report an improvement in tear film osmolarity following ocular lubricant use, and suggested that the OcuSense TearLab™ osmometer provides clinicians with the ability to pick up more subtle changes to the ocular surface than is possible with traditional dry eye diagnostic tests. However, our repeatability and reproducibility data suggest that these subtle changes may not be clinically relevant.

Another study investigated the use of OcuSense TearLab™ osmometer measurements as a potential marker of hydration status and reported statistically significant reductions in tear film osmolarity with fluid restriction [14]. The reported change was from 293±9 mOsms/l to 305±13 mOsms/l, which again falls within our measurement noise values and suggests that this change may not be clinically relevant.

Tomlinson and colleagues [15] compared the OcuSense TearLab™ osmometer with the freezing point depression Clifton Osmometer and found good correlation between the two instruments (r=0.904, p=0.006). However, their OcuSense TearLab™ osmometer readings were 308± 6 mOsm/l and 321±16 mOsm/l for the control and dry eye groups, respectively. The difference between these two groups falls within our reported measurement noise value. The difference between OcuSense TearLab™ osmometer readings for subjects with dry eye and primary Sjögren's syndrome (301.9±11.40 mOsm/l) and controls (294.85± 8.33 mOsm/l) also fell within our instrument noise value in a study of tear osmolarity in Sjögren's syndrome. [16] Similarly, Versura and colleagues report OcuSense TearLab™ osmometer values of 296.5±9.8 mOsm/l in normal eyes compared to 314.4±10.0 mOsm/l in people with severe dry eye [17]. According to our results, this difference could be considered to be caused by measurement noise.

Our results support the findings of Khanal and Miller [18] who report that consecutive OcuSense TearLab™ osmometer tear osmolarity readings in an individual can vary by up to 35 mOsms/l. They also found that an average of three readings is a reliable indicator of tear osmolarity at the 95% confidence level, but concluded that the variation in readings would make it difficult to use this instrument in the diagnosis of mild dry eye [18]. Similarly, Messmer et al. report that tear film osmolarity testing using the OcuSense TearLab™ osmometer did not discriminate between patients with DES (308.9±14.0 mOsm/l) and the control group (307.1± 11.3 mOsml/l) [19].

Many of the previous studies described above were performed on participants with DES. It is known that inter-individual variation in people with DES is significantly higher compared to healthy controls. Our repeatability data put in to question the clinical relevance of the results from these studies.

Our findings inform practitioners about the level of change over time that can be considered clinically relevant in healthy subjects. This value is 33mOsms/l; any change less than this should be considered to be due to measurement noise.

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Authorship All named authors were involved in the conception and design of the study, interpretation of data, and revising it critically for important intellectual content. MA, HB and FE were involved with the data analysis. All named authors gave final approval of the version to be published. MA collected the data. HB, MA and FE drafted the article. FE provided training with data collection.

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