121

Improved technique of vapour-pressure osmometry for smal tear volumes

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Purpose: We found the Wescor 5500 vapour-pressure osmometer gave irregular and anomalously high results with small tear samples (less than 2ul), and this seemed to depend on the lens-tissue discs supplied for the 2ul sample cup. We tested several disc materials for evenness and accuracy of performance.

Methods: 3mm discs were punched from lens tissue (LT), Whatman No. 1 paper (WP) and Millipore 0.2um filters (MP). The Wescor was calibrated using each disc type, and a 290 mOsm/kg standard was used throughout, for sample sizes between 2.0ul and 1.0ul. Broken pieces of Whatman Anodisc 0.2um filter (AP) of similar size were also tested.

Results: The mean reading for each paper type was 290 mOsm/kg but standard deviations for 2.0ul samples were: LT, ±23.1; WP, ±8.3. On reducing sample size, SD for LT remained high at ±19.5 for 1.5ul and ±24.3 for 1.0ul, while WP showed a SD of ±16.8 at 1.5ul rising to ±19.4 at 1.0ul. MP showed an extremely small SD of ±3.0 at 1.5ul rising to ±16.3 at 1.0ul, while AP gave an SD of ±29.0 at 1.0ul. If calibrated using WP, mean values using LT rose as sample size was reduced: 325, 385 and 376 mOsm/kg at 2.0, 1.5 and 1.0ul respectively.

Conclusions: The variation in errors with sample volume suggests that the Wescor Osmometer must be calibrated using the correct paper disc type. Use of LT tends to give higher results, but these may still be within the range of calibration readjustment of the instrument except at sample sizes below 1.0ul. The fibre distribution in individual LT discs suggests that LT has too variable a structure, and MP would be a better standard disc material. Anodisc is too friable for easy handling.

122

P2 receptors increase tear lysozyme levels in New Zealand white rabbits

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Purpose: Analyse the effect of UTP, Ap4A and INS365 on lysozyme levels in order to know if these substances can increase the amount of this protein in the tear film.

Methods: Lysozyme amount was obtained by measuring the inhibitory halos around a Whatman nº1 disc of 5 mm soaked in rabbit tears along 6 hours. Discs were put on petri dishes with Agar medium where Micrococcus Lysodeitikus bacteria was grown.

Results: All the tested substances have revealed an augment of tear lysozyme in rabbits. The results show a percentage of increase over the levels of basal lysozyme of 88% for UTP, 135% for Ap4A and 12,5% for INS365. Antagonists studies suggest that this effect is mediated through P2 receptors. The P2 antagonist receptors, suramin and PPADS, partially antagonize the effect of UTP and Ap4A respectively.

Conclusions: These substances could enhance the bactericidal activity of tear film by means of increasing the tear lysozyme protein in New Zealand white rabbits.

123

Where does ARK-700A actually measures peripheral corneal curvature? A comparison with MEDMONT videokeratoscope

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Purpose: Modern auto-refractors are available in most eye-care clinics, some of them including the ability to obtain central and peripheral measurements of corneal curvature as well, auto-keratometers (AK). The aim of this study was to find a correlation between peripheral measurements taken with the AK and videoqueratoscope (VKS).

Methods: Central and peripheral corneal curvature and eccentricity were measured in orthogonal meridians with AK and VKS in 122 eyes from 61 universitary students (23±4 years or age). Peripheral curvature obtained with VKS at 2.0/2.25/2.5/2.75 and 3.0 mm were compared with peripheral measurements taken with AK to find the best correlation between both instruments. The narrower 95% confidence interval (95%CI) and the closer to zero mean difference (mean diff.) were used as criteria.

Results: VKS give higher values for corneal eccentricity (0.56±0.10) than AK (0.48± 0.10) being significantly different from zero ($p<0.001$). Point to point analysis display closer agreement at 3,0 mm superior (mean diff.=0.28; 95%CI =±0.27), 3.0 mm inferior locations (mean diff.=0.19; 95%CI =±0.22), 2.5 mm temporal (mean diff.=-0.04; 95%CI =± 0.22) and 2.75 mm nasal (mean diff.=0.15; 95%CI =±0.35).

Conclusions: The application of peripheral curvature measurements with AK is limited and not comparable with VKS measurements. However, consistency of measurements makes AK a valuable tool to detect peripheral curvature variations in many clinical situations.

124

The development of tear sampling and bioassay techniques for the study of contact lens induced variations in tear protein profiles

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Purpose: This study has been particularly concerned with the development of techniques to enable the monitoring of specific tear proteins such as lipocalin as well as providing an overview of changes in more commonly monitored proteins. The effects of contact lens wear and diurnal variation are examined with the aim of providing an insight into causes of contact lens induced discomfort and particularly end of day effects.

Methods: Tear samples were collected by conventional microcapillaries, and two further techniques. The first used the lens in a novel way as a probe to sample a tear envelope (as distinct from tear products deposited on the lens). The second involved heating the lens in an extraction solution for 3 hours at 90°C to remove the protein bound or associated with the lens - both on and within the matrix. Samples were subjected to one dimensional SDS-PAGE. Gels stained in Coomassie blue or subjected to a modified Western Blotting technique.

Results: A great deal of information collected using electrophoretic techniques is of limited value because of the difficulty in processing, analysing rationalising and storing data.

Conclusions: We have been able to exploit improvements in software to produce processed data of a high standard, which has made it possible to follow changes in individual patients and assess changes in target proteins within large groups of patients very effectively. This poster describes the techniques involved and illustrates their use in patient monitoring.