

Protein and Microbial Determinations on Worn Contact Lenses Cleaned Conventionally Using the Lens2[®] Automatic Lens Cleaner

(Penentuan Protein dan Mikrobial ke Atas Kanta Sentuh Terpakai yang Dibersihkan Secara Konvensional Menggunakan Pembersih Kanta Sentuh Automatik Lens2[®])

HALIZA ABDUL MUTALIB, AHMAD ROHI GHAZALI
& NOOR SUHAILAH ALI

ABSTRACT

The accumulation of tear film proteins as well as microbes colonization onto worn contact lenses can be eliminated conventionally by mechanical rubbing during the cleaning process. Lens2[®] functions in rotation manner to loosen the deposits on the contact lens and has antimicrobial coating to keep lenses away from contamination. The objective of this study was to determine the efficiency of Lens2[®] to remove deposited protein and reduce microbial contamination compared to conventional method. Twenty-eight subjects each wore a pair of contact lens FDA Group 1 (Polymacon, SoftLens[®]38, Bausch & Lomb) for one month and cleaned them using multipurpose solution (COMPLETE[®] MoisturePLUSTM, Advanced Medical Optics) separately using two different methods. The right lens was cleaned conventionally while the left lens were cleaned using the Lens2[®]. The control group of thirteen subjects each wore a pair of contact lens for the same period and cleaned both conventionally. These lenses and its cases were then analyzed for protein deposition using Bichinchoninic Acid Assay (BCA) Kit (Sigma, USA) in 96-well plate. Microbial contamination was determined by culturing the samples on nutrient agar for bacteria and fungi and non-nutrient agar for amoeba isolation. The mean of total protein on control lenses ($17.014 \pm 13.246 \mu\text{g/mL}$) was not significantly different from those on the Lens2[®] ($21.623 \pm 19.127 \mu\text{g/mL}$). There were also low growth numbers of amoeba in each group of samples. Interestingly, there were no growths of amoeba from all Lens2[®] samples collected. There was also low growth numbers of bacteria in each sample group whereby Lens2[®] had the lowest growth of bacteria. No growth of fungi was obtained from all samples. The automatic lens cleaner, Lens2[®] was found to be as efficient as the conventional cleaning method. However, the Lens2[®] has additional advantage

because of its antimicrobial material and need shorter time in the cleaning process as well as easy and effective.

Key words: Contact lens, Protein deposit, Microbe, Lens2®

ABSTRAK

Pengumpulan protein daripada air mata dan juga mikrob pada kanta sentuh boleh disingkirkan secara konvensional iaitu dengan cara menggosok ketika proses mencuci. Alat Lens2® berfungsi secara berputar dengan tujuan melonggarkan deposit yang melekat pada kanta sentuh dan dibina dengan lapisan antibakteria untuk memastikan alat sentiasa bebas dari mikrob. Tujuan utama kajian ini adalah untuk menentukan keberkesanan alat Lens2® dalam membersihkan kanta sentuh daripada pengumpulan protein dan mengurangkan kontaminasi mikrob berbanding kaedah konvensional. Seramai dua puluh lapan subjek memakai sepasang kanta sentuh kumpulan 1 FDA (Polymacon, SoftLens®38, Bausch & Lomb) selama sebulan dan mencuci kanta dengan larutan serbaguna (COMPLETE® MoisturePLUS™, Advanced Medical Optics) secara berasingan menggunakan dua kaedah berbeza. Kanta sentuh kanan dicuci secara konvensional manakala kanta kiri dicuci menggunakan alat Lens2®. Kumpulan kawalan seramai tiga belas subjek yang turut memakai sepasang kanta sentuh selama sebulan dan mencuci kedua-dua belah kanta secara konvensional. Kanta-kanta tersebut kemudian dianalisis menggunakan Asai Bichinchoninic Acid (BCA) Kit (Sigma, USA) untuk menentukan pengumpulan protein. Kontaminasi mikrob ditentukan melalui pengkulturan sampel di atas agar nutrien untuk isolasi bakteria dan fungus manakala agar bukan-nutrien digunakan untuk isolasi amoeba. Min protein total di atas kanta sentuh ($17.014 \pm 13.246 \mu\text{g/mL}$) adalah tidak berbeza secara signifikan berbanding dengan nilai min protein total di atas Lens2® ($21.623 \pm 19.127 \mu\text{g/mL}$). Terdapat juga sedikit pertumbuhan amoeba bagi setiap kumpulan sampel. Walau bagaimanapun, tiada pertumbuhan amoeba diperolehi dari sampel alat Lens2®. Terdapat juga sedikit pertumbuhan bakteria bagi setiap kumpulan di mana alat Lens2® mempunyai pertumbuhan paling rendah. Tiada pertumbuhan fungus diperolehi pada semua kultur sampel. Keberkesanan alat pencuci kanta sentuh automatik, Lens2® didapati berfungsi sama efektif dengan kaedah mencuci konvensional. Namun, alat Lens2® mempunyai kelebihan materialnya yang anti mikrob dan juga dapat mempercepatkan proses pencucian kanta sentuh secara mudah dan berkesan.

Kata kunci: Kanta sentuh, Deposit protein, Mikrob, Lens2®

INTRODUCTION

The interaction of proteins with contact lenses and microbial contamination of the contact lenses, are important to contact lens wearers because these factors

could cause allergic, inflammatory reactions and infection. Much improvement has been done to produce safe contact lens wear. However, until now the risk has not been reduced while more cases of adverse reactions were reported due to contact lens wear (Mohamed Kamel et al. 2000; Seal 2003; Wynter-Allison et al. 2005).

The deposition of residual tear film molecules especially protein onto hydrogel contact lenses has been well-documented as an early phenomenon after a foreign material is inserted into a biological environment (Wollensak et al. 1990 & Lord et al. 2006). Oxidation, heat, ultraviolet (UV) light exposure and drying can denature the proteins. The denatured protein tends to bind or attach to other substances. The uncontrolled adsorption and accumulation of proteins on the surface of contact lenses, can lead to adverse reactions (Kingshott et al. 2000). Indeed, denatured proteins have been found to constitute a major portion of the deposits that accumulate on hydrogel contact lenses (Baker & Tighe 1981 & Tripathi & Tripathi 1980) which can lead to an increased potential for more serious ocular reactions such as giant papillary conjunctivitis (GPC) and superior limbic keratoconjunctivitis (SPK). However, in some instances, these protein deposits can be removed using cleaning techniques (Lord et al. 2006).

The clinical performance of the 'no-rub' multipurpose solution (MPS) has been repeatedly tested over the years. Differences in lysozyme residual and corneal staining detected with various MPS were significant. The efficiency in removing deposits from contact lenses does not depend solely on the mechanical rubbing but also on type of solution used (Steigemeier et al. 2004). Furthermore, not all 'no-rub' contact lens solutions were shown capable of removing protein deposit from soft contact lenses as claimed by the manufacturers (Mok et al. 2004).

Adverse responses that occur from contact lens use can also be produced as a consequence of microbial colonization of the lens which are frequently caused by microbial contamination of the contact lens surface (Solomon et al. 1994; Sankaridurg et al. 1996; Holden et al. 1996; Sankaridurg et al. 1999). Contact lens contamination commonly occurs through hand contact (Mowrey-McKee et al. 1992), from the eyelids of wearers and from environmental sources (Willcox et al. 1997). One of the initial steps in the development of the microbial caused adverse responses is the binding of microbes to a contact lens (Willcox et al. 2001). Type of material of the contact lenses (Cook et al. 1993a; Cook et al. 1993b; Fleiszig et al. 1996) and the deposits on contact lenses especially protein play an important role in providing a conducive environment for microbes adhesion. Subsequent to adhesion, it is likely that microbes will further colonize the lens surfaces by growing on the surfaces (Willcox et al. 2001). Many contact lens users ignore the advice of their contact lens practitioner and lens care instructions and rinse their lenses or storage case in tap water, which may introduce pathogenic microbes especially *Acanthamoeba* to the storage case. Microbes can potentially transfer from the storage case to the cornea by the

contact lens. The lens will hold the microbes in place on the eye, which may ultimately lead to infection.

There are various techniques of lens rubbing that has been recognized. Lenses are commonly rubbed using the index finger in circular motion, radially on the palm or rubbing it between the index finger and the thumb. Some wearers with rough fingers could unintentionally damage their contact lenses by rubbing with their fingers. In many instances wearers clean their lenses by manually shaking the lens casing. The technique used by wearers, if not done properly will introduce accumulation of deposit at the midperiphery area of the lens. The deposit accumulated at these areas will start an allergy response when there is friction or contact between lens and palpebral aperture during blinking.

Lens2® (GREEN H.T Co., Ltd.) is an automatic lens cleaner. It has been marketed worldwide since 7 years ago but it is still poorly recognized in Asia. It claimed to be very convenient since it is portable, compact in design and auto-cleaning within 3 minutes. Both contact lenses can be cleaned separately simultaneously. It is also claimed that the Lens2® is able to remove any protein on the lens (including hard lens), clean and store the lens without touching them. Interestingly, an antimicrobe (Biocleanact™) was added into the raw material make-up of the lens cleaner, to prevent contamination and colonization of microbes on contact lenses due to storage. It also helps to prevent secondary contamination by hand by providing special vacuum lens holder for contact lens fitting.

The existence of this automatic lens cleaner is not only giving solution to the contact lens care problems in terms of time and hygienic factors, it also indirectly educates and creates awareness to contact lens wearers of the importance of practicing proper and hygienic lens care. It is recognized that some of the risk factors of adverse effects associated with contact lens wear may be modified, and should be addressed by the design of contact lens materials and lens care products (Liesegang 1997). This would serve to protect both the eyes of contact lens wearers, and to reduce the burden (in terms of both cost and time) upon primary healthcare.

The purpose of the present study was to determine protein deposition and microbial contamination on contact lenses cleaned with automatic lens cleaner, Lens2® and conventional method. So far, there are no studies that analyze the effects of different cleaning method on protein deposit and microbial activity.

EXPERIMENTAL METHODS

Lens2® is a battery operated lens cleaner machine which contain an antimicrobe basket to hold a pair of contact lenses separately. The basket is filled with multipurpose solution and rotates with high speed for three minutes.

New monthly disposable contact lenses FDA Group 1 (Polymacon, SoftLens®38, Bausch & Lomb) were given to forty-one subjects. This lens was

chosen as it is the most commonly used by the public and easily available in Malaysia. Subjects were divided into two groups whereby twenty-eight subjects each wore a pair of contact lens for 1 month and cleaned them separately as instructed. For the right lens, the lens was cleaned conventionally (manually rub) while for the left lens, Lens2[®] was used. The other thirteen subjects wore the same brand of contact lenses for the same period and cleaned both lens conventionally (control group). The lenses and all the storage cases of Lens2[®] were then analyzed for protein deposition and microbial contamination. All controls and subjects used multipurpose solution (COMPLETE[®] MoisturePLUS[™], Advanced Medical Optics). All lenses, lens casings and Lens2[®] were returned after 1 month of full wear for laboratory investigation. Samples were separated into 4 different categories. Sample CLC were contact lenses cleaned conventionally, sample CLL were contact lenses cleaned using the Lens2[®] and later transferred to a normal casing, sample CSC were the normal casing used to store the control lenses and the sample CSL in the anti bacteria lens casing from the Lens2[®].

ISOLATION OF AMOEBA

Each contact lens was placed into a universal bottle containing 3 mL transport media i.e. PAGE Amebic Saline (PAS). The conventional cases was swabbed using sterile cotton swab which then was placed into another universal bottle contain PAS. Each PAS was vortexed it was filtered using membrane filter. The membrane filter was removed and inverted onto non-nutrient agar (NNA) plate containing 1 mL of heat-killed *Escherichia coli* suspension which served as food for acanthamoeba. Lastly, the plates were sealed with parafilm and incubated at 30°C for 3 days.

On the third day, the filter membrane was taken out from NNA plate and the plate was observed under inverted light microscope daily. The existence of trophozoites with contractile vacuole which constrict every 30-60 seconds or/ and the existence of cysts with fine endocyst and ectocyst indicate the existence of *Acanthamoeba* spp. Plates with no trophozoite or/and cyst until day 14 were considered negative.

ISOLATION OF BACTERIA AND FUNGUS

About 0.5 mL of PAS which contained a swab from each sample was spread on nutrient agar (NA) plate and then incubated for 72 hours at 37°C.

PROTEIN DETERMINATION

Each of the same lenses that had been used for isolation of microbes was incubated in 1.5 mL of extraction solvent consisting of a 50:50 mix of 0.2%

trifluoroacetic acid and acetonitrile for 24 hours at room temperature in the dark to remove protein from worn contact lens (Keith et al. 2003). The extraction then was assay for protein using Bichinchoninic Acid Assay (BCA) Kit (Sigma, USA). Standard curves were prepared from at least five different protein concentrations of the standard protein during each test assay.

STATISTICAL TEST

Student-*t* Test was performed to compare the cleaning efficiency of both techniques in removing deposited protein. A *p*-value less than or equal to 0.05 is reported as a statistically significant difference. The growth of microbes was calculated by the percentage of number of plates contaminated.

RESULTS

AMOUNT OF PROTEIN THAT REMAINED ATTACHED TO CONTACT LENSES AFTER TWO MODES OF CLEANING

The mean total protein removed from control lenses cleaned conventionally and using the ALCM Lens2[®] was $17.014 \pm 13.2 \mu\text{g/mL}$ and $21.623 \pm 19.1 \mu\text{g/mL}$ respectively. The difference was not significant.

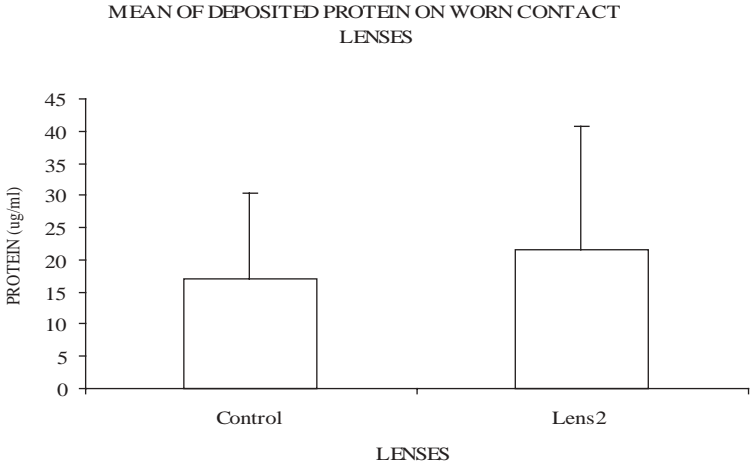


FIGURE 1. Comparison of mean deposited protein on contact lenses that cleaned conventionally and with Lens2[®], an automatic lens cleaner. Values were presented in mean \pm SD (standard deviation).

AMOEBA

Table 1 shows low isolation rate of amoeba in 4 groups of samples and no isolation of amoeba on all Lens2® samples. However, the lenses cleaned using the Lens2® when transferred to the normal casings showed 7.1% of amoeba contamination. Swabs of the normal casings which were used to house the contact lens also gave the same percentage. The lens casings of the control lenses also showed a higher percentage of amoeba isolation compared to the lens itself.

ISOLATION OF BACTERIA AND FUNGI

Table 2 shows bacteria were isolated from all sample groups. However, Lens2® had the lowest growth. No growth of fungus was observed in all samples.

TABLE 1. Number and percentage of sample plates with amoeba isolation

Sample type	CLC	CLL	CSC	CSL	Lens2®
Proportion of positive samples	1/54 (1.9%)	2/28 (7.1%)	2/54 (3.7%)	2/28 (7.1%)	0/28 (0)

CLC : Contact lenses that were conventionally cleaned (Control)

CLL : Contact lenses that were cleaned using Lens2®

CSC : Lens cases for control lenses

CSL : Lens cases for Lens2® lenses

TABLE 2. Number and percentage of sample plates with bacterial isolation

Sample type	CLC	CLL	CSC	CSL	Lens2®
Proportion of positive samples	30/54 (55.6%)	14/28 (50%)	33/54 (61.1%)	20/28 (71.4%)	8/28 (28.6%)

CLC : Contact lenses that were conventionally cleaned (Control)

CLL : Contact lenses that were cleaned using Lens2®

CSC : Lens cases for control lenses

CSL : Lens cases for Lens2® lenses

DISCUSSION

The cleaning efficiency of Lens2® was found not significantly different to the conventionally cleaning method. Both techniques were effective and efficient in removing deposited protein and reducing microbes' contamination on worn

contact lenses. However, there are many advantages of Lens2® that are useful and beneficial to contact lens wearers that could attract user to choose Lens2® to help them practice proper and hygienic care of their lenses. As it is auto-functional, the contact lens cleaning process is easier and time saving. In addition, both contact lenses can be cleaned simultaneously. It is very convenient since it is portable, compact in design and small in size that allows for easy packing when traveling. Lens2® is also equipped with mirror and special vacuum lens holder (Spoid), which makes the putting of the lens easier and with less handling thus reducing the risk of microbes infection since contact lens contamination occurs commonly through hand contact (Mowrey-McKee et al. 1992). In addition, it can help reducing the risk of lens tear, scratch and chip during cleaning process. It can be ideal for children and youngster who are unable to clean their lens conventionally themselves.

Amoeba Keratitis such as *Acanthamoeba* Keratitis is most severe and potentially sight threatening ocular parasitic disease and is recognized as the most challenging among ocular infections because of the protracted painful clinical course and frequently encountered treatment failures (Mohamed Kamel et al. 2000). Not all commercial solutions for contact lenses have the parasitidal activity needed against *Acanthamoeba* sp. (Borazjani & Kilvington 2005) Moreover, many acanthamoebae were reported to be resistant to disinfectants, temperature variation and desiccation (Walker 1996). The isolation rate of amoeba from all samples groups were very low or nil. Lens2® sample indicates effectiveness of the conventional method and the multipurpose solution in reducing the contamination of amoeba on those samples.

Samples marked with Lens2® have the lowest isolate rate of bacterial contamination. No growth of fungus was observed in all samples. It proved that the antimicrobial agent (Biocleanact™) that was added into the raw material of Lens2® was effective in reducing contamination of bacteria and hence the risk of getting ocular bacterial infections. The growth of bacteria observed from the contact lenses that were cleaned using Lens2® might have originated from the airborne contamination while transferring the lenses into the cases. Moreover, it is quite impossible to keep equipments 100% free from microbes contamination as they are everywhere in the environment and even on human body such as the eyelids as normal microbiota (Borazjani & Kilvington 2005; Khunkitti et al. 1998). In addition, type of material of the contact lenses and the deposits on contact lenses especially protein play an important role in providing a conducive environment for microbes adhesion (Willcox et al. 2001; Cowell et al. 1998; Taylor et al. 1998). Subsequent to adhesion, it was likely that microbes will further colonize on the lens surfaces by growing on that lens surface itself (Willcox et al. 2001).

There are a lot of factors that could contribute the variability in our results. A larger number of subjects is actually needed to reduce the variability and any

probabilities which could influence the results. Although the ratio of samples number and control in this study was almost 2:1 which is statistically good study power, it still needed more subjects to give more accurate results. By having a larger number of subjects, it is also easy to take out the factors such as polymorphism factors and diseases which can lead to false finding. A limitation of this study was that no exact instructions to the subjects on the method of cleaning the lenses. This is important since different people have different ways of cleaning their lenses. There was also a rare possibility that the subjects who used Lens2® did not follow the correct instruction on ways to use it. In addition, the microbes determination in this study did not identify the isolated microbes. Therefore, we could not differentiate pathogens from normal microbes. The risk of contamination was high as it was difficult to keep equipments 100% free from microbes contamination (Khunkitti et al. 1998; Borazjani & Kilvington 2005). Besides, there was a possibility for the samples to get microbes contamination while collecting and delivering from the subjects to the laboratory and while culturing the samples.

CONCLUSION

Lens2® has equal efficacy to the conventional cleaning method. The antimicrobial lens casing was effective in controlling microbial contamination. Since the lens casings were the main source of infection in lens wear, serious attention should not be placed only on the cleaning method but also in lens storing.

REFERENCES

- Baker, D. & Tighe, B. J. 1981. Polymers in contact lens applications VIII. The problem of biocompatibility. *Contact Lens J.* 10: 3-14.
- Borazjani, R. N. & Kilvington, S. 2005. Efficacy of multipurpose solution against *Acanthamoeba* species. *Contact Lens & Anterior Eye* 28: 169-175.
- Cook, A. D., Sagers, R. D. & Pitt, W. G. 1993a. Bacterial adhesion to protein coated hydrogels. *J. Biomater. Appl.* 8: 72-89.
- Cook, A. D., Sagers, R. D. & Pitt, W. G. 1993b. Bacterial adhesion to poly (HEMA)-based hydrogels. *J. Biomed. Mater. Res.* 27: 119-126.
- Cowell, B. A., Willcox, M. D. & Schneider, R. P. 1998. A relatively small change in sodium chloride concentration has a strong effect on adhesion of ocular bacteria to contact lenses. *J. Appl. Microbiol.* 84: 950-954.
- Fleiszig, S. M., Evans, D. J., Mowrey-Mckee, M. F., Payor, R., Zaidi, T. S., Vallas, V., Muller E. & Pier, G. B. 1996. Factors affecting Staphylococcus epidermidis adhesion o contact lenses. *Optom. Vis. Sci.* 73: 590-594.
- Holden, B. A., La, H. D., Grant, T., Newton-Howes, J., Baleriola-Lucas, C., Willcox, M. D. & Sweeney, D. F. 1996. Gram-negative bacteria can induce contact lens related acute red eye.

- Khunkitti, W., Lloyd, D., Furr, J. R. & Russell, A. D. 1998. *Acanthamoeba castellanii*: growth, encystment, excystment and biocide susceptibility. *J. Infect.* 36: 43-48.
- Kingshott, P., St John, H. A. W., Chatelier, R. C., Griesser, H. J. 2000. Matrix-assisted laser desorption ionization mass spectrometry detection of protein adsorbed in vivo onto contact lenses. *J. Biomed. Mater. Res.* 49: 36-42.
- Liesegang, T. J. 1997. Contact lens-related microbial keratitis: Part I: epidemiology. *Cornea* 16: 125-131.
- Lord, M. S., Stenzel, M. H., Simmons, A. & Milthorpe, B. K.. 2006. The effect of charge groups on protein interactions with poly(HEMA) hydrogels. *Biomaterials* 27: 567-575.
- Mohamed Kamel, A.G., Faridah Hanum, Norazah, A., Noor Rain, A., Hay, J. & Seal, D. 2000. A case of waterborne contact lens associated *Acanthamoeba* keratitis from Malaysia : Successful treatment with Chlorhexidine and Propamidine. *Int. Med. J.* 7: 63-65.
- Mok, K. H., Cheung, R. W., Wong, B. K., Yip, K. K. & Lee, V. W. 2004. Effectiveness of no-rub contact lens cleaning on protein removal: A pilot study. *Optom. Vis. Sci.* 81(6): 468-470.
- Mowrey-McKee, M. F., Monnat, K., Sampson, H. J., Smith, C. M., Davies, G. A., Mandt, L. & Proskin, H. M. 1992. Microbial contamination of hydrophilic contact lenses. Part I : quantitation of microbes on patient worn-and-handled lenses. *CLAO J.* 18: 87-91.
- Sankaridurg, P. R., Sharma, S., Willcox, M. D., Sweeney, D. F., Naduvilath, T. J., Holden, B. A. & Rao, G. N. 1999. Colonization of hydrogel lenses with *Streptococcus pneumoniae*: risk of development of corneal infiltrates. *Cornea* 18: 289-295.
- Sankaridurg, P. R., Willcox, M. D., Sharma, S., Gopinathan, U., Janakiraman, D., Hickson, S., Vuppala, N., Sweeney, D. F. & Rao, G. N. 1996. *Haemophilus influenzae* adherent to contact lenses associated with production of acute ocular inflammation. *J. Clin. Microbiol.* 34: 2426-2431.
- Seal, D. V. 2003. *Acanthamoeba* keratitis update - Incidence, molecular epidemiology and new drugs for treatment. *Eye* 17: 893-905.
- Solomon, O. D., Loff, H., Perla, B., Kellis, A., Belkin, J., Roth, A. S. & Zucker, J. 1994. Testing hypotheses for risk factors for contact lens-associated infectious keratitis in an animal model. *CLAO J.* 20: 109-113.
- Taylor, R. L., Willcox, M. D., Williams, T. J. & Verran, J. 1998. Modulation of bacterial adhesion to hydrogel contact lenses by albumin. *Optom. Vision Sci.* 75 : 23-29.
- Tripathi, R. C. & Tripathi, B. J. 1980. The pathology of soft contact lens spoilage. *Ophthalmology* 87: 365-380.
- Willcox, M. D. P., Harmis, N., Cowell, B. A., Williams, B. A. & Holden, B. A. 2001. Bacterial interactions with contact lenses; effects of lens material, lens wear and microbial physiology. *Biomaterials* 22: 3235-3247.
- Willcox, M. D., Power, K. N., Staphleton, F., Leitch, C., Harmis, N. & Sweeney, D. F. 1997. Potential sources of bacteria that are isolated from contact lenses during wear. *Optom. Vision Sci.* 74: 1030-1038.

- Wollensak, G., Mur, E., Mayr, A., Baier, G., Göttinger, W. & Stöffler, G. 1990. Effective methods for the investigation of human tear film proteins and lipids. *Graefes. Arch. Clin. Exp. Ophthalmol.* 228(1): 78-82.
- Wynter-Allison, Z., Lorenzo Morales, J., Calder, D., Radlein, K., Ortega-Rivas, A. & Lindo, J. F. 2005. Acanthamoeba infection as a cause of severe keratitis in a soft contact lens wearer in Jamaica. *Am. J. Trop. Med. Hyg.* 73(1): 92-94.

Haliza Abdul Mutalib
Department of Optometry
Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur

Ahmad Rohi Ghazali
Noor Suhailah Ali
Department of Biomedical Sciences
Faculty of Allied Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz
50300 Kuala Lumpur

Corresponding author: haliza@medic.ukm.my
Received: June 2007
Accepted for publication: November 2007