PARASITOLOGY

# Effectiveness of Commercially Available Contact Lens Disinfecting Solutions against *Acanthamoeba* spp. Clinical Isolates

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## ABSTRACT

***Introduction:*** **Contact lens wearers are at highest risk of contracting *Acanthamoeba* keratitis which is caused by the invasion of the free-living amoeba on the cornea. Concern arises as commercially available contact lens disinfecting solutions are not required to prove its effectiveness against *Acanthamoeba* cysts.**

***Objective:*** **The main purpose of this study is to investigate the effectiveness of few selected multipurpose contact lens disinfecting solutions available in the market on three clinical isolates of *Acanthamoeba*.**

***Methods:*** **The *Acanthamoeba* clinical isolates were coded as HS 60, HS 69 and HS 74 which originated from corneal scrapings. The testing was done according to the recommended soaking time by the manufacturers at 4 and 6 hours and also prolonged soaking at 8 and 24 hours. The *Acanthamoeba* spp. isolates were classified based on morphological groups I, II, and III. Three brands of commercially available contact lens solutions; Opti Free® Express®, Complete® Multi-Purpose Solution and Renu® Fresh™, were evaluated. Contact lens disinfecting solution efficacy test was performed by adding 100 ul of the cyst suspension to 1ml of contact lens solutions and incubated at manufacturer's recommendation time of 4, 6, and testing time of 8 and 24 hours. After each soaking time, 100 ul of the sample tested was placed onto the non-nutrient agar seeded with heat-killed *E. coli* and incubated at 30°C (± 2°C) and observed daily for 14 days under an inverted microscope to detect the presence of trophozoites.**

***Results:*** **Opti Free® Express®, Complete® Multi-Purpose Solution, and Renu® Fresh were ineffective at disinfecting all three *Acanthamoeba* isolates. All of the isolates were identified to be under Group II (Polyphagids).**

***Conclusion:*** **All 3 multipurpose contact lens disinfecting solutions tested, were not effective at disinfecting *Acanthamoeba* cyst isolates and might not provide protection for its users against *Acanthamoeba* keratitis**

## KEY WORDS

***Acanthamoeba*, multi-purpose contact lens disinfecting solution, Malaysia**

## INTRODUCTION

*Acanthamoeba* is one of the opportunistic protozoans that is widely distributed in the environment and well known to produce serious infection to human which include keratitis that may result in permanent visual impairment. *Acanthamoeba* is a small, free living amoeba that can be found in the air, soil and aquatic environments. It is classified into three major groups based on the morphological features of the cysts. Group I has large cysts with rounded outer walls (ectocysts) that are clearly separated from the inner walls (endocysts). Group II cysts are smaller, with variable endocyst shapes while Group III cysts are smaller than Group II cysts, with poorly separated walls (Pussard & Pons, 1977).

In Malaysia, the first case of *Acanthamoeba* keratitis was reported in 1995 by Mohamed Kamel & Norazah, involving a female contact lens wearer. Subsequently, more cases were seen though not reported and by the end of 2001, at least 10 cases were diagnosed at UKM alone (Kamel *et al*. 2003). Since then, *Acanthamoeba* keratitis cases were on the rise, in line with the popularity of contact lens usage. Mohamed Kamel *et al*. 2018a reported that *Acanthamoeba* spp. were successfully isolated from 14.8% (11/74 cases) of corneal scraping samples from keratitis patients. This study showed that all of the *Acanthamoeba* keratitis patients were contact lens wearers, majority of whom were women (90.9%). Haliza *et al*. 2005 reported that amongst the risk factors for contracting *Acanthamoeba* keratitis were the use of tap water to wash the contact lens, wearing contact lens while swimming, cleaning contact lens using normal saline without disinfectant and extended wear of contact lenses. Thus, it is very important for us to identify the commercially available contact lens disinfecting solutions that can disinfect the *Acanthamoeba* cyst and prevent the occurrence of *Acanthamoeba* keratitis.

There are basically two groups of contact lens disinfecting/cleaning solutions available for use with soft lenses. The first group is the multipurpose disinfecting solutions which contain biguanides in one form or another or polyquaternium-1. They may also contain other disinfectants such as myristamidopropyl dimethylamine. All these agents have the same general mode of action which is to cause disruption of the microbial membranes that ultimately leads to death of the microbes (FDA,

Received on January 10, 2021 and accepted on March 17, 2021

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**Table 1: Contact lens disinfecting solutions (clds) tested and their properties**

| Brand | Manufacturer | Disinfection system | Type of contact lens | Recommended soaking time | Active ingredient (s) |
|---|---|---|---|---|---|
| Complete® Multi Purpose Solution | Abbot Medical Optics Inc | MPS | Soft | 6 hours | 0.0001% polyhexamethylene biguanide |
| Renu® Fresh™ | Bausch & Lomb | MPS | Soft | 4 hours | DYMED™ (Polyaminopropyl biguanide) 0.0001% |
| Opti-Free® Express® | Alcon Laboratories Inc | MPS | Soft | 6 hours | 0.001% polyquaternium-1 0.0005% myristamidopropyl dimethyl-amine |

MPS - Multi-Purpose Solution

**Table 2: Effectiveness of Complete®, Renu® freshTM, and Opti-Free® Express® clds towards *Acanthamoeba* spp. isolates.**

| Isolates | 4 Hours* | 6 Hours* | 8 Hours | 24 Hours |
|---|---|---|---|---|
| HS 60 | + | + | + | + |
| HS 69 | + | + | + | + |
| HS 74 | + | + | + | + |

Key:

\+ Trophozoite present (clds not effective)

\- Trophozoite absent (clds effective)

* Recommended soaking hours by manufacturer

**Table 3: Results for Positive & Negative Control Tests**

| Isolates | Cysts suspension | 3% $H_2O_2$ | PAGE solution | Disinfecting Solutions |
|---|---|---|---|---|
| HS 60 | + | - | x | x |
| HS 69 | + | - | x | x |
| HS 74 | + | - | x | x |

Key :

\+ Trophozoite present (cysts viable)

\- Trophozoite absent (3% $H_2O_2$ effective)

x Absence of trophozoites and cysts (no contamination)

**Table 4: Classification for the *Acanthamoeba* spp. isolates based on the cyst morphological characteristics.**

| *Acanthamoeba* spp. isolates | Group |
|---|---|
| Clinical isolate - HS 60 | Group II |
| Clinical isolate - HS 69 | Group II |
| Clinical isolate - HS 74 | Group II |

2015). The second group is essentially one entity namely hydrogen peroxide ($H_2O_2$). $H_2O_2$ is a strong oxidising agent and can disrupt many aspects of a microbe and acts on various proteins, lipids and DNA leading to cell death (Hughes *et al*. 2003).

In Malaysia, most of the reported cases of *Acanthamoeba* keratitis are associated with contact lens wear. Thus, proper selection of contact lens disinfecting solutions among the users is very important in order to overcome any infection towards the eyes by any types of microorganisms including *Acanthamoeba* spp.

## MATERIALS AND METHODS

### Source of Acanthamoeba spp.

*Acanthamoeba* spp isolates were obtained from the *Acanthamoeba* Lab, Department of Parasitology, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM). Three isolates from the clinical subculture (HS 60, HS 69 and HS 74) were used in this study. All isolates were subcultured onto Non-nutrient agar seeded with *E. coli* and incubated for 10 days to ensure all of the trophozoites had transformed into cyst stage.

### Multipurpose contact lens disinfecting solutions

Three brands of commercially available multipurpose contact lens disinfecting solutions ie; Opti Free® Express® (Alcon, USA), Complete® Multi-Purpose Solution (AMO, USA) and Renu® Fresh™ (Bausch & Lomb, USA) were used in this study. Their chemical ingredients and properties are shown in Table 1.

### Sample processing and testing

The test conducted was a modified version based on culture-filtration technique by Narasimhan et al. 2002. The contact lens disinfecting solutions efficacy test, was carried out in appendorf tubes. 1 ml of the contact lens solutions was placed in each of the appendorf tube. Then 100 ul of the cyst suspension containing about 1 x $10^5$ cysts was pipetted into each tube containing the disinfecting solution. The tubes were stored at room temperature at durations following the soaking intervals of 4 hours, 6 hours, 8 hours and 24 hours.

Positive and negatives controls were carried out alongside the test samples. Two types of positive controls were used. The first positive control was the cysts suspension with PAGE saline solution, used to validate the viability of the cysts, where the cyst can develop into trophozoites without the presence of the inhibitor. The second positive control was the cysts suspension with 3% of hydrogen peroxide. Two types of negative controls were used comprising PAGE saline solution and the contact lens disinfecting solutions. This was done to ensure that the solutions were free from contaminants.

After each soaking time, 100 ul of the sample tested was placed onto the non-nutrient agar seeded with heat-killed *E. coli* and incubated at 30°C (± 2°C) for 3 days. Observation was made daily for 14 days to identify the presence of *Acanthamoeba* spp. trophozoites. The presence of *Acanthamoeba* trophozoites was recorded as positive while its absence was recorded as negative.

## RESULTS

All three clinical isolates were resistant towards all multipurpose contact lens solutions tested (Complete®, Renu® fresh and Opti-Free® Express®) and showed no anti-*Acanthamoeba* activity at the recommended soaking hours as well as the extended time (Table 2). The first positive control was the cysts suspension with the PAGE saline solution which showed viability of the cysts as they were able to develop into trophozoites (Table 3). The second positive control was the cysts suspension with 3% of hydrogen peroxide. The result showed, the absence of trophozoites in all samples tested after being immersed in 3% hydrogen peroxide (Table 3). Two types of negative controls were used comprising PAGE saline solution and all three contact lens disinfecting solutions used in this study showed no contaminations (Table 3). For morphological classification, isolates HS 60, HS 69 and HS 74 belong to Group II with the cysts showing smaller size from the Group I and represent variable endocyst shapes (Table 4).

## DISCUSSION

Without early detection and proper treatment, *Acanthamoeba* keratitis can cause ulceration of the cornea which in turn leads to blindness. The treatment is usually difficult and treatment failure is not uncommon and therefore any risk should be avoided as a preventive measure. *Acanthamoeba* keratitis is known to occur mainly amongst the contact lens wearers, therefore the choice of a good contact lens disinfecting solution capable of disinfecting *Acanthamoeba* cysts is very important to the users.

Opti Free® Express® multipurpose contact lens solution containing POLYQUAD® (Polyquaternium-1 0.001%) and ALDOX® (Myristamidopropyl dimethylamine (MAPD) 0.0005%) as active ingredients failed to disinfect all three isolates used in this experiment in all tested soaking hours. This finding is in parallel with results from previous study conducted by Niszl and Markus in 1998 where Opti Free® Express® multipurpose contact lens solution also failed to kill the *Acanthamoeba* strains used.

Kilvington *et al*. (2002) discovered that Myristamidopropyl dimethylamine (MAPD) demonstrated cysticidal effect against *Acanthamoeba* strains recalcitrant to medical therapy at a minimum cysticidal concentrations of 6.25 to 25 µg/ml. At concentration of 10 to 30 µg/ml it gave at least a 3-log cyst kill after 6 hours exposure. The concentration of MAPD in Opti-Free Express disinfecting solution is much lower than the minimum cysticidal concentration and therefore fails to show cysticidal effect against all isolates of *Acanthamoeba* cysts. Our previous study (Mohamed Kamel *et al*. 2018b) also showed that Opti Free® Express® multipurpose contact lens solution failed to exhibit anti-*Acanthamoeba* activity when tested against 4 clinical isolates of *Acanthamoeba*.

Complete® Multi-Purpose Solution also failed to exhibit anti-*Acanthamoeba* activity. All cyst isolates tested successfully transformed into trophozoites at all soaking hours including the prolonged exposure of 8 and 24 hours. PHMB (Polyhexamethylene biguanide 0.0001%) is the active ingredient present in Complete® Multi-Purpose Solution. This concentration is well below the concentration which is effective and well tolerated at 200 mg/L (0.02%) when used as treatment of patients with keratitis caused by *Acanthamoeba* spp (Larkin *et al*. 1992). Niszl and Markus (1998) also found that Complete Multi-Purpose Solution did not inactivate cysts of any of the strains of *Acanthamoeba* tested. Viable cysts were detected in all experiments after 7 days of exposure to the solution. Similar findings were reported by Shoff *et al*. (2007) and our previous studies (Mohamed Kamel *et al*. 2016a, 2016b, Mohamed Kamel *et al*. 2020)

There was no anti-Acanthamoeba activity exhibited by Renu® Fresh™ either. The active ingredient in this multipurpose solution is DYMED containing Polyaminopropyl biguanide (PAPB) 0.0001% and Poloxamine 1%. Niszl and Markus, 1998 discovered that Polyaminopropyl biguanide (PAPB) at higher concentration of 0.0005 mg/ml, was found to be totally ineffective against *Acanthamoeba* cysts. At even higher concentration of 0.0015%, PAPB was found to be ineffective against *Acanthamoeba* cysts by Connor *et al*. 1991. Our previous studies (Mohamed Kamel *et al*. 2015 & 2016a) also demonstrated that Renu® Fresh™ was ineffective against *Acanthamoeba* cysts.

On the classification of the *Acanthamoeba* isolates, morphological characteristics of the cysts (Pussard & Pons, 1977) showed that all three isolates were categorized under Group II (Polyphagids) which was known to exhibit pathogenicity. It is therefore not so surprising to see that they are quite resistant to most antimicrobials and disinfecting solutions.

## CONCLUSION

Most commercially available contact lens disinfecting solutions in the market are not effective at disinfecting *Acanthamoeba* cysts, as shown in this study. None of the three multipurpose contact lens disinfecting solutions showed anti-*Acanthamoeba* activity even at extended soaking hours. Further studies need to be conducted in search for the more effective contact lens disinfecting solutions that possess anti-*Acanthamoeba* activity that can help prevent *Acanthamoeba* infection of the cornea, among the contact lens wearers.

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