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# PLANT-BASED THERAPY: THE PROSPECTIVE THERAPEUTIC COMPOUNDS AGAINST *ACANTHAMOEBA* INFECTIONS

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Malaysia

INDY/PULSE

# THE EYE-FEASTING PARASITE THAT GETS IN THROUGH YOUR CONTACT LENSES

## Parasite in contact lens leaves woman blind, bald and paralysed

38 tablets daily

Published September 20, 2017

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Before and after infection photos of victim of contact lens infection, Mrs. Claire Wilkinson. Mail photo

A woman has been left blinded, bald and paralysed by a parasite lurking in her contact lenses.

Claire Wilkinson, 38, from Brisbane, Australia, has battled the *acanthamoeba keratitis* (AK) parasite for a

# Acanthamoeba Ate my Eyeballs

A student lost sight in one eye after failing to remove her disposable contact lenses for six months. So how did tabloid journalists turn it into such a popular news story?

By Roisin Mcguigan

Why did a case report of a patient with acanthamoeba keratitis become one of the most well-read stories on the world's most-visited newspaper website, MailOnline? The patient, a 23-year old

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## Winter Weather Advisory

# After water skiing spill, man gets amoeba in eye and can't see, until....

By Kaysey Price on May 3, 2017 at 4:53 p.m.

19

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# I was blinded by my contact lens

By Claire Bates  
BBC News

12 April 2017

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# ba: A Dangerous Pathogen

organism, how it causes keratitis and how patients can avoid

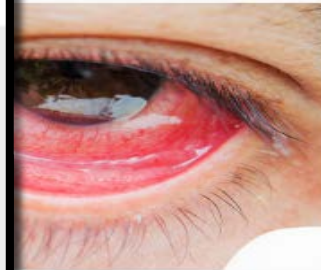


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# ns a Water Ride Gave Him an parasite

horrifying as you'd think



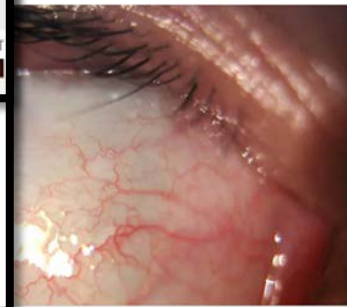
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Why Doctors Will No Longer

splash is one of the best parts of a water innocent fun, though: One man from at a splash from a water ride at an



Author



Hany Elsheikha  
Associate Professor of Parasitology, University of Nottingham

Disclosure statement

Hany Elsheikha is affiliated with The European Scientific Council for Companion Animal Parasitology (ESCCAP) of UK & Ireland.

Partners



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## Kerana Nak Cantik, Wanita Kongsi Ma Buta Gara-Gara Pakai Contact Lens

Ditulis pada 3 January 2017 • 0 Komen • 853 Paparan

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86  
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Kerana Nak Cantik, Wanita Kongsi Matanya Hampir Buta Gara-Gara Pakai Contact Lens



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## Mata dijangkiti parasit

Oleh TAUFIK SALIMIN



DETIK cemas dilalui Nur Masyitah Atiqah Amran, 26, tatkala bangun dari tidur apabila mata kirinya bengkak, kemerahan dan berair.

## Teacher loses partial vision after getting eye water



Nik Nur Adibah Athirah

Nik Nur Adibah Athirah Timeline Recent

Add Friend Follow

Nik Nur Adibah Athirah added 7 new photos.  
4 January at 17:03

Sebelum ni baca kisah orang , kali ni terkena kat diri sendiri . Awal 2018 yg agak menyedihkan.

29/12/17 - pakai lens , dalam 2 3 jam lepas tu tiba2 rasa sakit , tapi tak dapat nak buang lagi sebab ada kat luar . Sampai rumah lebih kurang pukul 8 , buang lens tu dan mata dah jd kabur . Mula2 tu ingat biasa , mgkin lepas tido nanti jd okey balik.

30/12/2017 - Nak pergi keje mata mata kabur lagi , pakai cermin mata pun kabur lg . Lebih kurang tengahari , mata sebelah kanan , f...  
Continue reading

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Photos

SERVE NOW

lens | Berhati-hati guna contact lens. Bagi yang suka dan kerap boleh janganlah kerap sangat walaupun nak nampak cantik. Mulah yang tercantik dan anugerah yang terindah dan tidak ternilai ang dikurniakan olehNya.

book Amy Syaquena yang kami kongsiikan melalui Pixelperfect.



## ta' sekejap



> Gara-gara mahu mata berwarna, seorang gadis nyaris hilang penglihatan akibat memakai kanta lekap RM25.

"Buta' sekejap gara gara

## MAHANA KANTA LEKAP MURAH

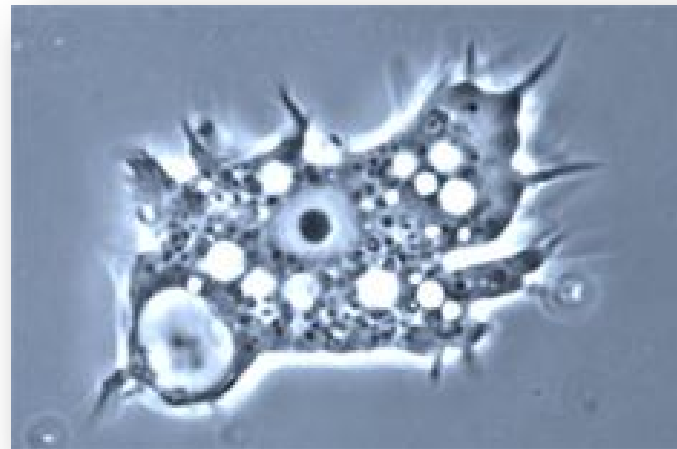
makai kanta lekap berwarna jenis murah dan tidak is berusia 18 tahun dari Kapar, di sini, kini berdepan hari selepas memakainya.

mbeli kanta lekap itu dengan RM25 sepasang di berkata sejurus bangun daripada tidur esoknya, dia bila kedua-dua mata sakit dan membengkak teruk.

# WHAT IS ACANTHAMOEBA?

**PROTOZOA** that are **OPPORTUNISTIC** pathogens in **HUMANS**.

Also known as **AMPHIZOIC AMOEBA** – ability to exist as **FREE-LIVING** organisms in **NATURE** and occasionally **INVADE** a **HOST** and live as **PARASITES** within **HOST TISSUE** (human).



Soil

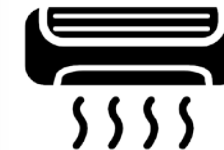


Dust

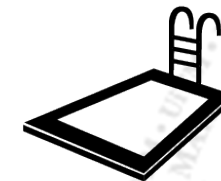


Tap Water

## DISTRIBUTIONS



Air  
Conditioning  
Unit



Swimming  
Pool



CONTACT  
LENS

# WHAT IS ACANTHAMOEBA?



Ubiquitously present in the environment and that we commonly encounter this organism in our routine lives!



Presence of anti-*Acanthamoeba* antibodies in up to 100% healthy populations in New Zealand.



More than 85% in individuals of London that came from different countries.

# WHAT IS ACANTHAMOEBEA?

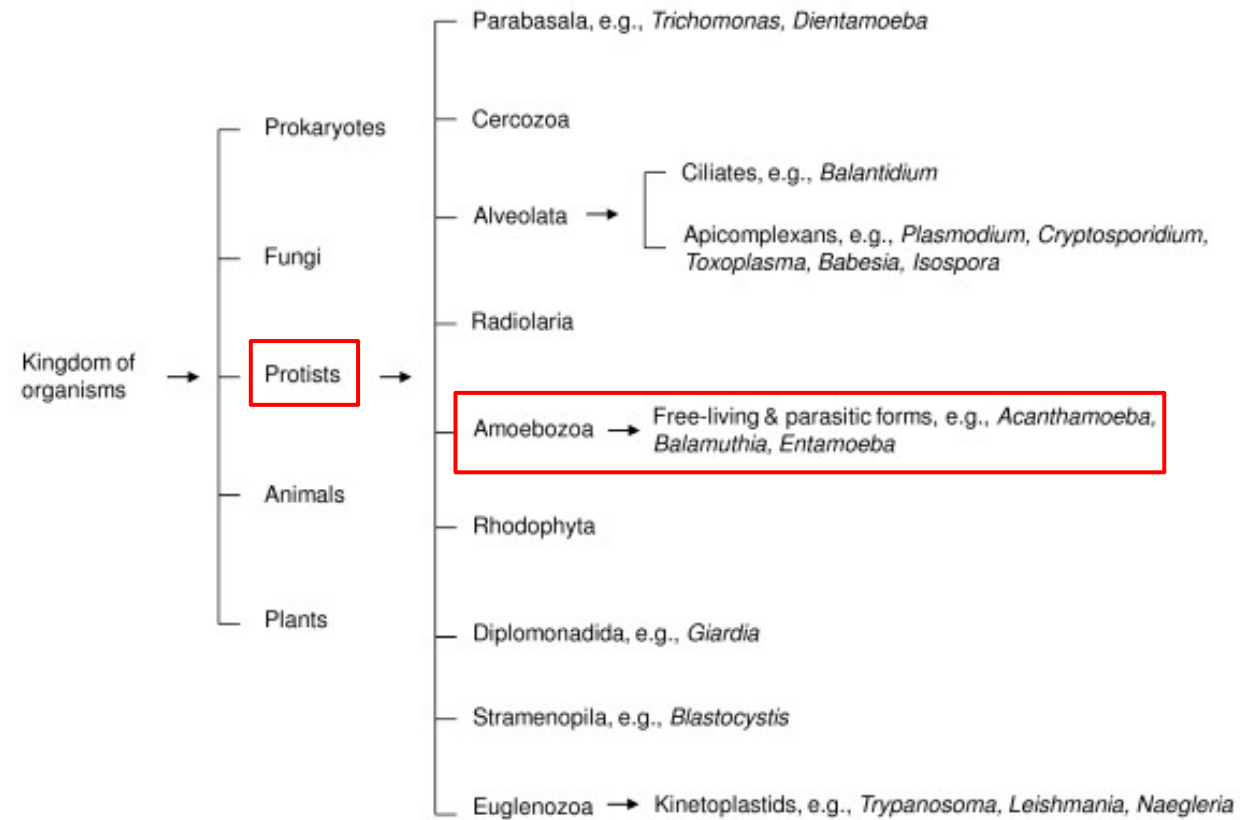
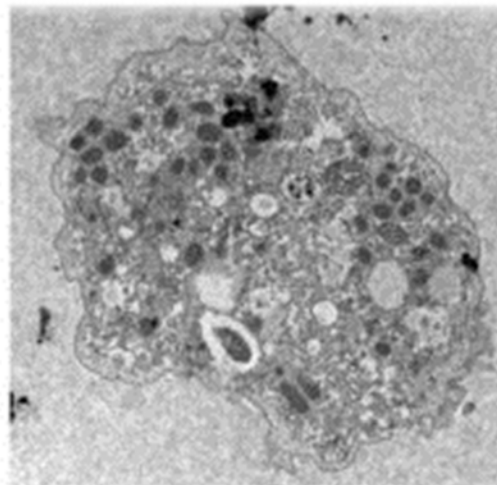


Figure 1. The classification of protists based on ribosomal rRNA sequences.

# MORPHOLOGICAL FORMS

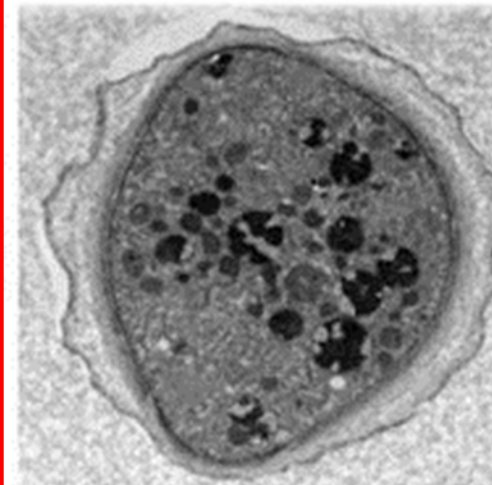


2μm

## TROPHOZOITE

- ❖ Feeding and Dividing
- ❖ Asexual
- ❖ Cyst Forming

Harsh conditions  
 →  
 ←  
 Favourable conditions



2μm

## CYST

- ❖ Response to Adversity
- ❖ Dormant, Resistant
- ❖ Double-Walled with Pores

# ACANTHAMOEBA CYST

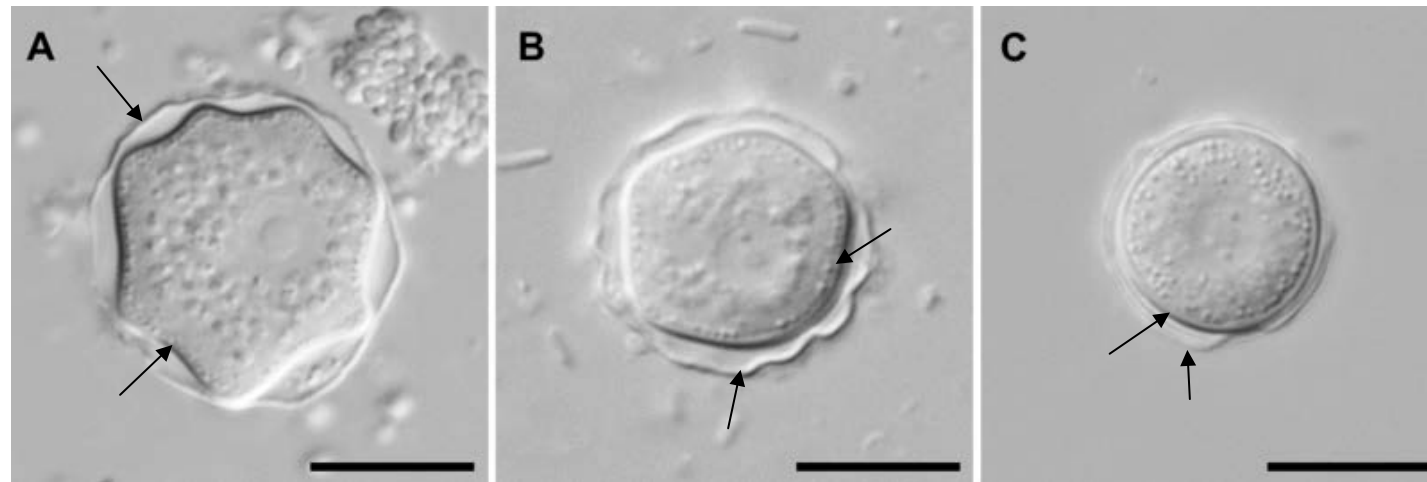
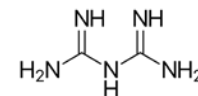


Figure 2. *Acanthamoeba* cysts in interference contrast microscopy. (A) morphological group I, (B) morphological group II and (C) morphological group III

- **OUTER WALL** – proteins and polysaccharides.
- **INNER WALLS** – cellulose.
- **RESISTANT** – extremes of temperature, pH, desiccation, irradiation and **ANTIMICROBIAL AGENTS**.
- Only 1 class of medication – **BIGUANIDES** have cysticidal activity.





# ACANTHAMOEBA GENOTYPING

<i>Acanthamoeba</i> genotypes	Human disease association
T1	Encephalitis
^T2a	Keratitis, Encephalitis
^T2b - ccap1501/3c-alike sequences	NA
T3	Keratitis
<b>T4*</b>	<b>Keratitis, Encephalitis</b>
T5	Keratitis, Encephalitis
T6	Keratitis
T7	NA
T8	NA
T9	NA
T10	Keratitis, Encephalitis
T11	Keratitis
T12	Encephalitis
T13	NA
T14	NA
T15	Keratitis
T16	NA
T17	NA

\*this genotype has been most associated with both diseases  
 ^basis of T2 division into T2a and T2b has been proposed by Maghsoud *et al.*, (2005)  
 NA - no disease association has been found yet

Each genotype exhibits 5% or more sequence divergence.

Table 1. Known *Acanthamoeba* genotypes and their associations with human diseases, e.g., keratitis and granulomatous encephalitis.

# DISCOVERY OF ACANTHAMOEBA

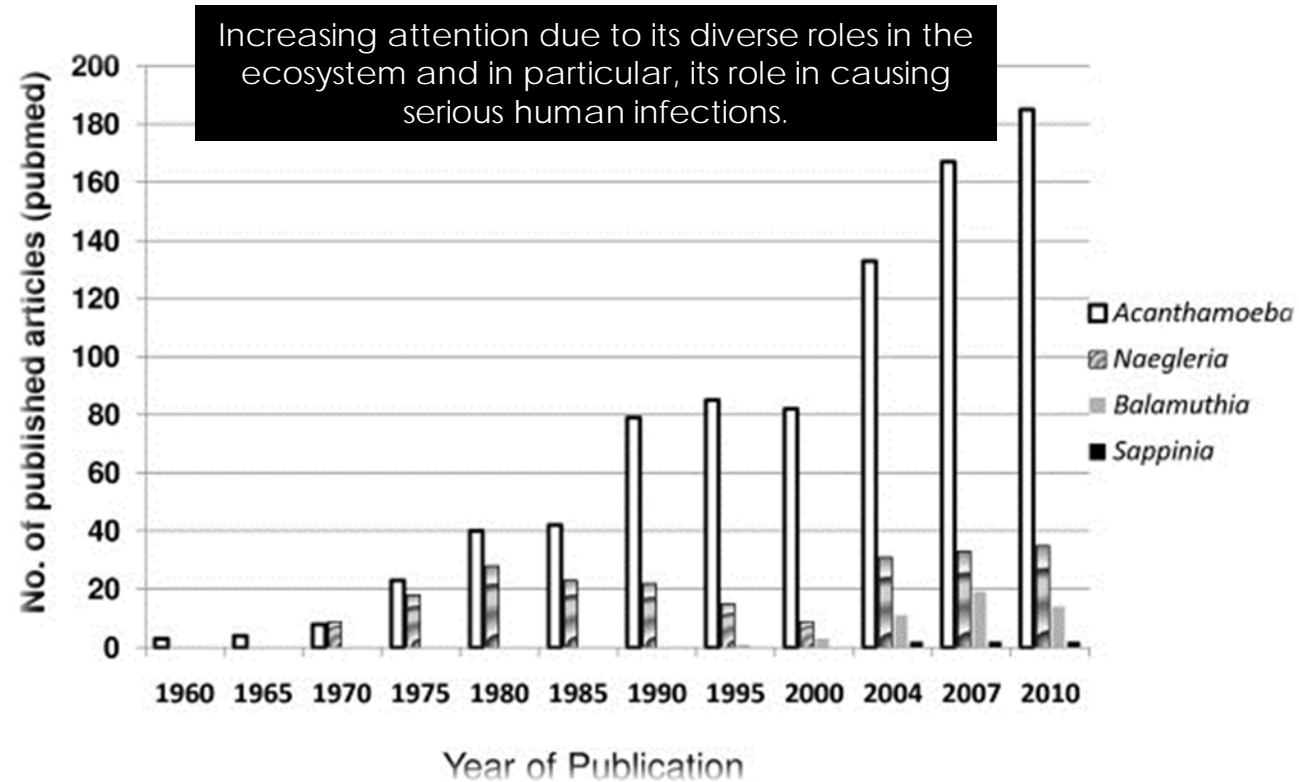


Figure 3. Increasing scientific interest in the field of *Acanthamoeba* as determined by published articles over the last five decades.

# DISCOVERY OF ACANTHAMOEBA

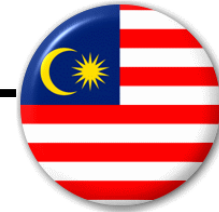
- *A. polyphaga* and *A. castellanii* – sight-threatening infection of the cornea (**ACANTHAMOEBA KERATITIS**).
- *A. culbertsoni* – life-threatening meningoencephalitis (**GRANULOMATOUS AMOEBIC ENCEPHALITIS**).
- Immunocompromised individuals – **CUTANEOUS** and **SINUS** diseases.



# ACANTHAMOEBA CASES IN SOUTHEAST ASIA

Disease	Country	Case/History
<i>Acanthamoeba</i> keratitis (AK)	Malaysia, Indonesia, Thailand and Singapore	Contact lens use
Granulomatous Amoebic Encephalitis (GAE)	Thailand	8 X, 3 √
	Malaysia	1 X
	Indonesia	1 X
<i>Balamuthia</i> Amoebic Encephalitis (BAE)	Thailand	1 X *23 year old healthy male after falling into a swamp during a motorbike accident in 2004.
Primary Amoebic Meningoencephalitis (PAM)	Thailand	10 X, 2 √
	Vietnam	2 √

# ACANTHAMOEBA CASES IN SOUTHEAST ASIA



*Neurology Asia 2018; 23(2) : 179 – 184*

## ***Acanthamoeba* encephalitis in an immunocompetent child and review of the imaging features of intracranial acanthamoebic infections in immunocompetent patients**

<sup>1,2</sup>Mohammad HANAFIAH, <sup>2</sup>Azura Mohamed Mukhari SHAHIZON, <sup>1,2</sup>Mohd Farhan HAMDAN, <sup>3</sup>Sau Wei WONG, <sup>3</sup>Yoganathan KANAHESWARI

<sup>1</sup>*Department of Radiology, Faculty of Medicine and Institute of Pathology, Medical & Forensic Laboratory, Universiti Teknologi MARA, Selangor; Department of <sup>2</sup>Radiology and <sup>3</sup>Pediatrics, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia*



# ACANTHAMOEBA CASES IN SOUTHEAST ASIA

## Amoebic meningoencephalitis in Samarinda East Kalimantan

**L R D Siagian<sup>1,2\*</sup>, V M L Toruan<sup>1,3</sup>, Y O Hutahaen<sup>4</sup>, C Gunawan<sup>5</sup>**

<sup>1</sup>Departement of Parasitology, Faculty of Medicine, Mulawarman University, Samarinda, Indonesia

<sup>2</sup> Department of Clinical Pathology, Faculty of Medicine, Mulawarman University, Samarinda, Indonesia

<sup>3</sup> Department of Dermato venereology, Faculty of Medicine, Mulawarman University, Samarinda, Indonesia

<sup>4</sup>Department of Neurology, Faculty of Medicine, Mulawarman University, Samarinda, Indonesia

<sup>5</sup>Departmen of Internal Medicine Faculty of Medicine, Mulawarman University, Samarinda, Indonesia



# ACANTHAMOEBA LIFE CYCLE

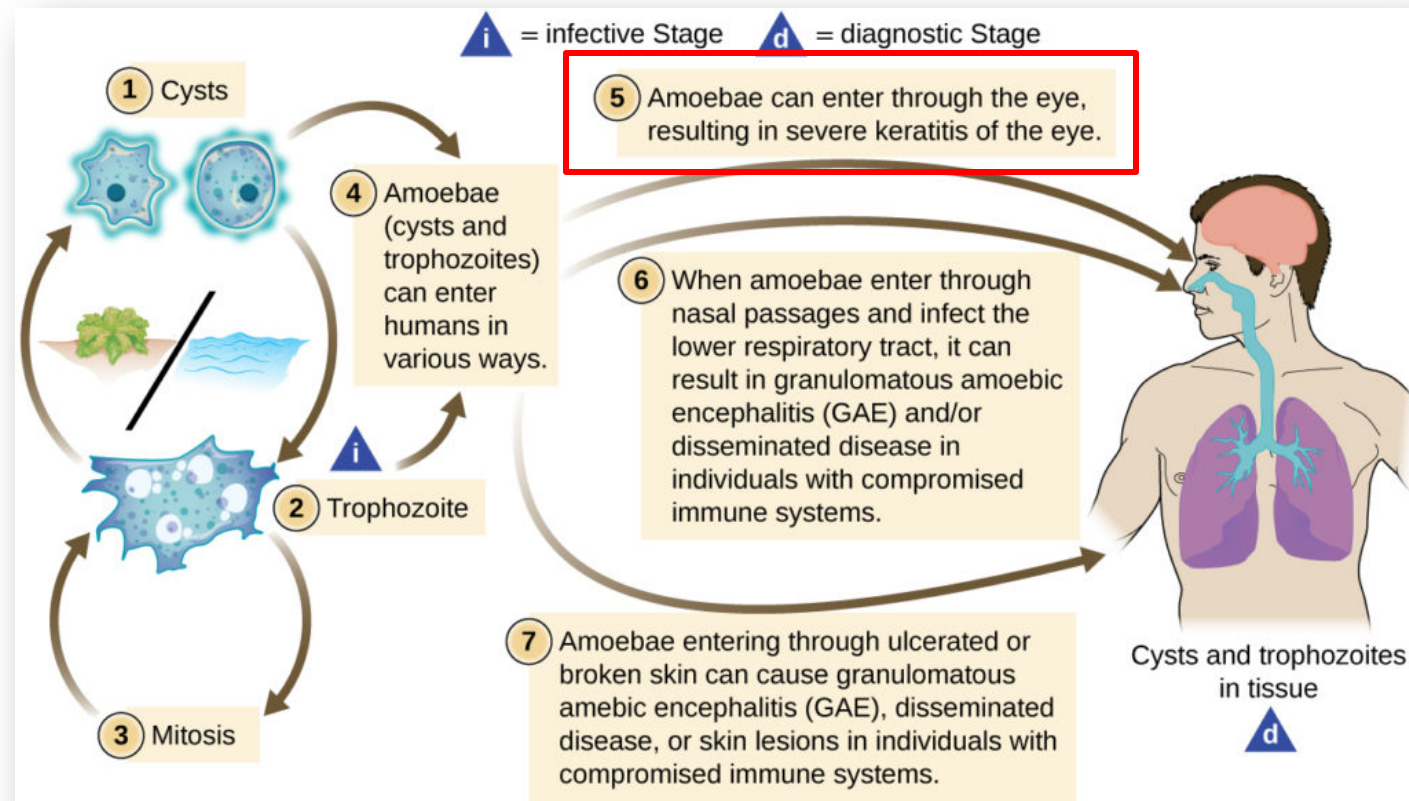


Figure 4. *Acanthamoeba* life cycle; cysts and trophozoites are both capable of entering the body through various routes.

# ACANTHAMOEBA KERATITIS

Uncommon eye infection  
– number of infected  
persons increased in  
developing countries.

Increase of AK cases is  
associated with the use of  
**CONTACT LENSES.**



First case reported in 1975  
– patient who sustained  
eye trauma outdoors.

Contact lens wearers –  
**HIGH RISK GROUP.**

<sup>4</sup>TU, E.Y. and Joslin, C.E. 2010. Recent outbreaks of atypical contact lens-related keratitis: what we have learned?  
Am J Ophthalmol, 150: 602-608.

<sup>5</sup>Lorenzo-Morales, J., Khan, N.A. and Walochnik, J. 2015. An update on *Acanthamoeba* keratitis: diagnosis,  
pathogenesis and treatment. Parasite, 22: 10.



# FIRST CASE OF ACANTHAMOEBA KERATITIS IN MALAYSIA

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (1995) 85

## Short Report

### First case of *Acanthamoeba* keratitis in Malaysia

A. G. Mohamed Kamel<sup>1</sup> and A. Norazah<sup>2</sup> <sup>1</sup>*Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, 50300 Jalan Raja Muda Abdul Aziz, Kuala Lumpur, Malaysia;* <sup>2</sup>*Division of Bacteriology, Institute for Medical Research, 50588 Jalan Pahang, Kuala Lumpur, Malaysia*

**Keywords:** *Acanthamoeba*, keratitis, Malaysia

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# PREVALENCE OF ACANTHAMOEBA IN MALAYSIA

2013:  
8%

66

International Medical Journal Vol. 20, No. 1, pp. 66 - 68 , February 2013

BIOMEDICAL SCIENCES

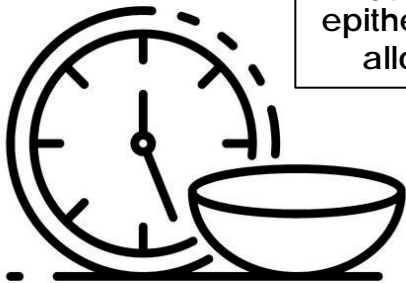
## Isolation of *Acanthamoeba* spp. from Contact Lens Paraphernalia

Mohamed Kamel Abd. Ghani<sup>1)</sup>, Saleha Abdul Majid<sup>1)</sup>, Noradillah Samseh Abdullah<sup>2)</sup>,  
Anisah Nordin<sup>3)</sup>, Yusof Suboh<sup>3)</sup>, Noraina Abd Rahim<sup>3)</sup>, Haliza Abdul Mutalib<sup>4)</sup>,  
Norazah Ahmad<sup>5)</sup>



# RISK FACTORS OF ACANTHAMOEBA

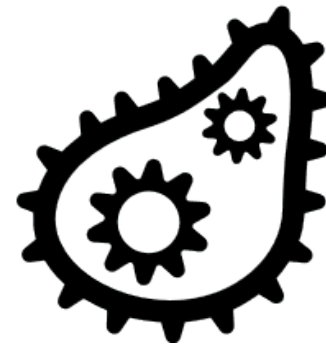
Induced hypoxic and hypercapnic state promoting epithelial cell desquamation and allowing microbial invasion.



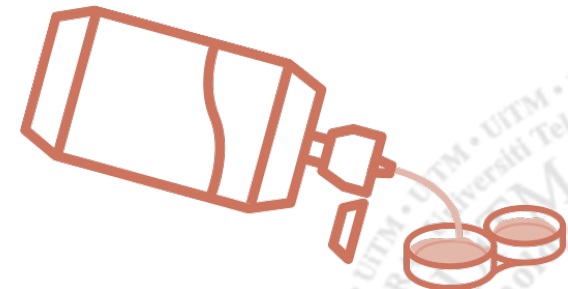
Contact lens wear for extended period of time



Lack of personal hygiene



Biofilm formation of contact lens



**INEFFECTIVE LENS  
DISINFECTION SYSTEM**

“The competition for more **COMFORTABLE** and **CONSUMER FRIENDLY** contact lens solutions have been identified as playing a key role in the increase in atypical causes of AK”.



# ACANTHAMOEBA SIGNS AND SYMPTOMS

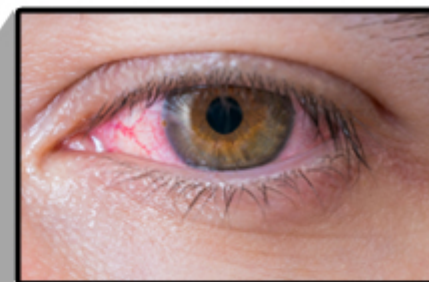


Pain in the Eye



Watering of Eyes

## Symptoms and Signs of *Acanthamoeba* Keratitis



Redness of the Eye



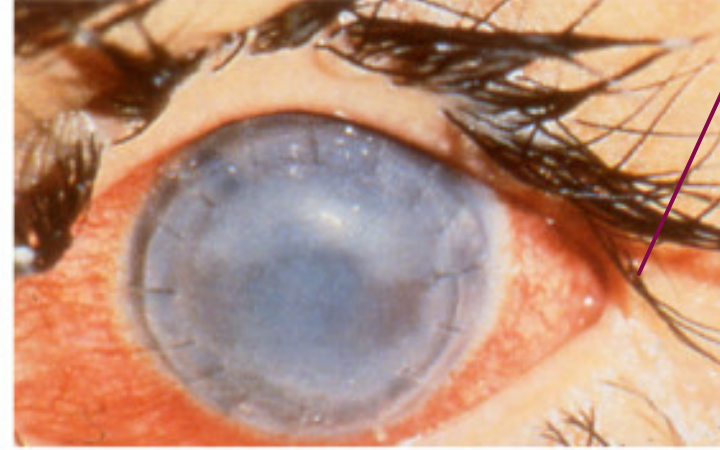
Photophobia

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# ACANTHAMOEBA SIGNS AND SYMPTOMS



Normal eye



*Acanthamoeba*-infected eye

Stromal findings which occur later include single or multiple stromal infiltrates or satellite lesions usually suggest AK.

Figure 5. (A) Normal eye and (B) Infected eye exhibiting recurrent *Acanthamoeba* infection following corneal transplant with severe corneal damage and loss of vision.



# PATHOGENESIS OF *ACANTHAMOEBA* KERATITIS

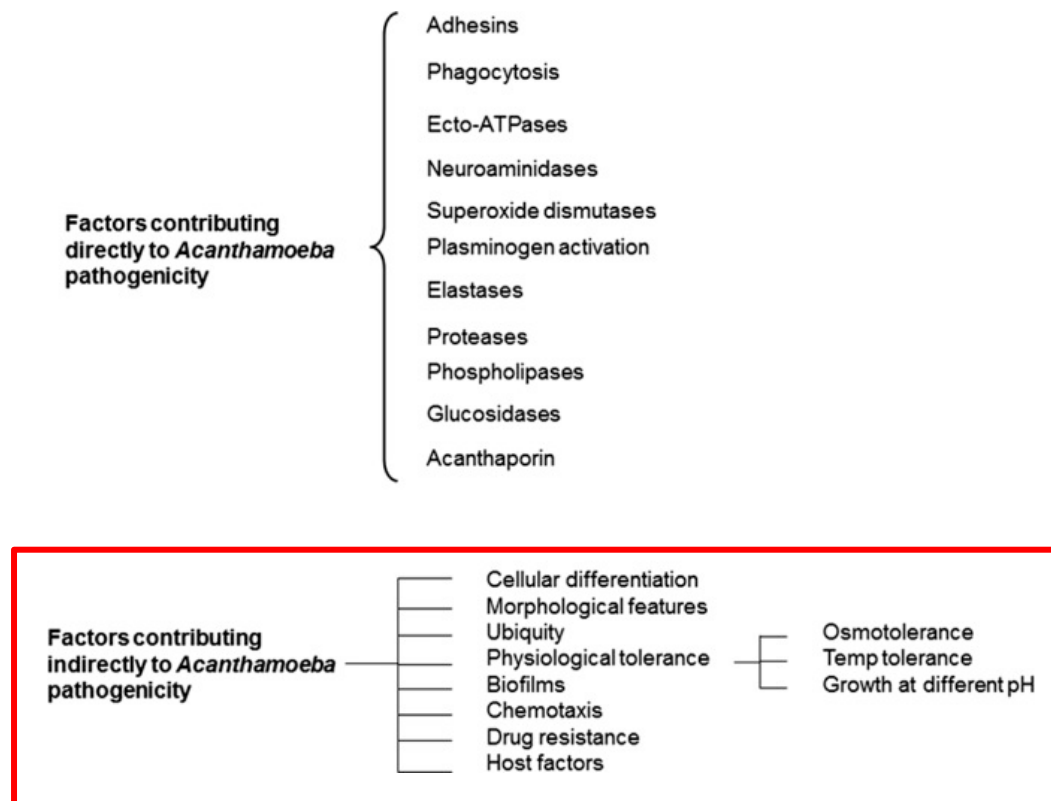


Figure 7. Factors contributing to the pathogenicity of *Acanthamoeba*.

# DIAGNOSTIC TECHNIQUES

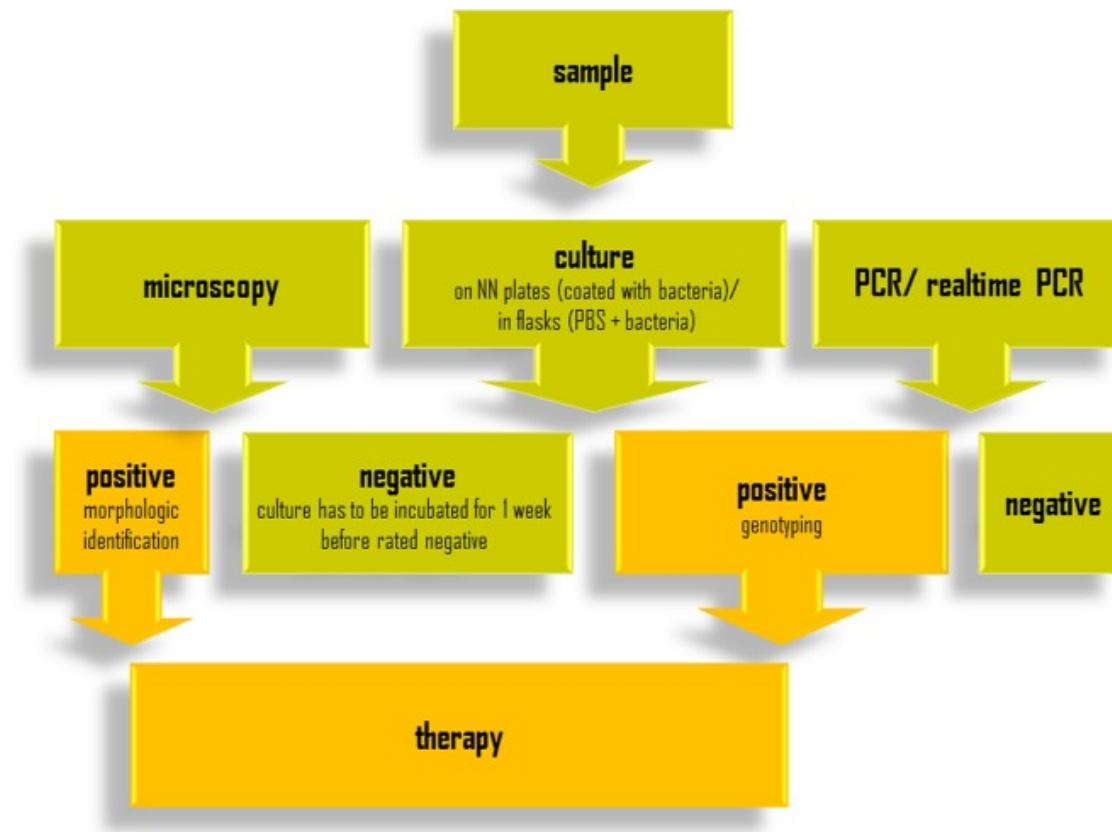


Figure 8. Overview of the diagnostic procedure for *Acanthamoeba keratitis*.



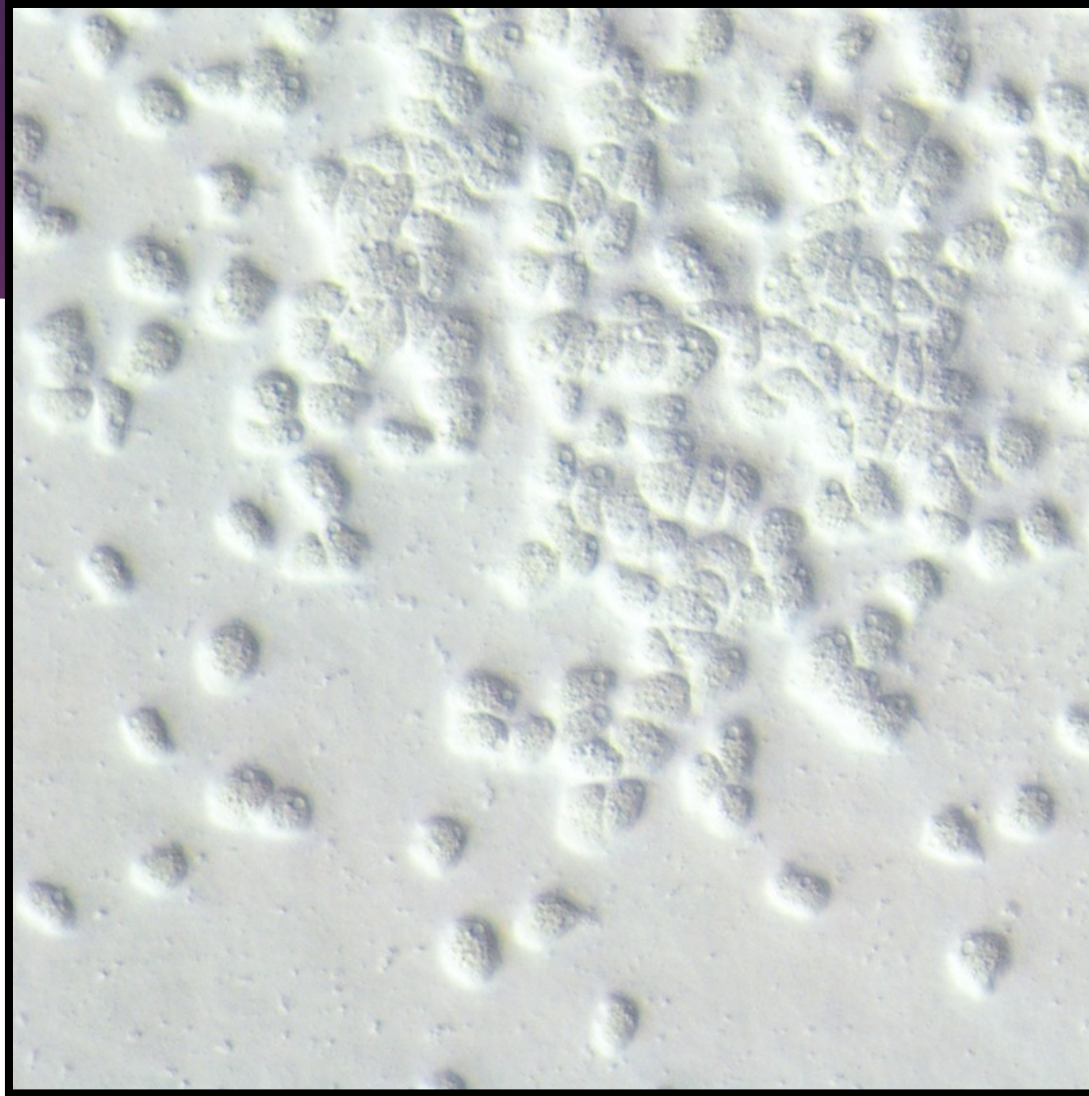


Figure 9. *Acanthamoeba* trophozoites observed in culture in a case of severe AK infection.

# CURRENT THERAPEUTIC APPROACHES

Group	Drugs	Doses Topical/Oral
Biguanides	Chlorhexidine	0.02%-0.2%
	Polyhexamethylenebiguanide	0.02%-0.06%
Diamidines	Propamidine isethionate	0.1%
	Hexamidine	0.1%
Azoles	Voriconazole	Oral 100 mg per day
Aminoglycoside	Neomycin	1.0%

Table 2. List of anti-*Acanthamoeba* drugs and doses.

<i>Acanthamoeba</i> spp.		
Life cycle	Two forms: trophozoite and cyst	
Morphological Features	Trophozoite: Vesicular nucleus; spine-like pseudopodia projecting from surface; cyst: wall with two layers	
<i>In vitro</i> cultivation	Axenic, bacterized, and defined media; tissue culture cells; growth at 37 °C (CNS isolates) or 30 °C (keratitis isolates)	
The most important diseases	<b>Granulomatous Amoebic Encephalitis (GAE)</b>	<b>Amoebic keratitis (AK)</b>
Incubation period	Weeks to months (GAE)	Days (AK)
High risk people	Typically Immune-compromised Individuals such as AIDS patients (GAE)	Mainly contact-lens wearers; Low secretory IgA may contribute (AK)
Clinical Characteristics	Headache, fever, nausea, vomiting, behavioral changes, stiff neck, lethargy, loss of consciousness, seizures, coma, and death (GAE)	Painful, sight-redness, photophobia, edema (AK)
Clinical course	Sub-acute course; acute stage fatal in weeks (GAE)	Penetration of amoebae into cornea; stromal ring due to PMN infiltrate (AK)
Laboratory diagnostic methods	Amoeba seen in CSF; Molecular method (GAE)	Not relevant (AK)
Neuroimaging (CT and/or MRI)	Presence of space occupying or ring enhancing lesion (GAE)	Corneal scrapings or biopsy; confocal microscopy; PCR (AK)
Prevention	Monitoring of environmental sources such as waters, ventilators, air conditioning units (GAE)	Use of anti-acanthamoeba lens solutions; avoiding swimming or bathing with contact lenses (AK)
Chemical therapy	Combination of drugs such as ketoconazole, fluconazole, itraconazole, azithromycin, sulfadiazine, amphotericin B, rifampin, voriconazole, and miltefosine (GAE)	Combination chemotherapeutic agents such as polyhexamethylene biguanide, chlorhexidine (AK)
Prognosis	Poor; diagnosis is often Post-mortem, only a few patients have survived (GAE)	Good with early diagnosis and proper treatment (AK)

Table 3. Characteristics of *Acanthamoeba* as an agents of amoebic encephalitis and amoebic keratitis.

## CURRENT ISSUES



“Recent studies have reported a significant increase in the number of AK patients in the     ”.

“At present, there are **>120 MILLION** people wearing contact lenses, thus there is a growing need to be aware of the associated risks”.



“This is particularly important in view of the **INEFFECTIVENESS OF CLEANING SOLUTIONS** of some contact lens products”.

# CURRENT ISSUES

RESEARCH

## National Outbreak of *Acanthamoeba* Keratitis Associated with Use of a Contact Lens Solution, United States

Jennifer R. Verani, Suchita A. Lorick, Jonathan S. Yoder, Michael J. Beach, Christopher R. Braden, Jacquelin M. Roberts, Craig S. Conover, Sue Chen, Kateesha A. McConnell, Douglas C. Chang, Benjamin J. Park, Dan B. Jones, Govinda S. Visvesvara, and Sharon L. Roy, for the *Acanthamoeba* Keratitis Investigation Team<sup>1</sup>



# MULTIPURPOSE CONTACT LENS DISINFECTING SOLUTION

MPDS – designed for **CLEANING, DISINFECTING, RINSING** and **STORING** using a single formulation.

Most of these solutions contains **PHMB** or **polyquad** (poly-quaternium-1).



Susceptibility of *Acanthamoeba* to MPDS was variable – depend on the **STAGE, TYPE & DILUTION** of disinfecting solution and **LENGTH OF EXPOSURE TIME**.

Ineffective against *Acanthamoeba* cyst. **RESISTANCE** to **BIOCIDES** during encystation has been observed.


## MULTIPURPOSE CONTACT LENS DISINFECTING SOLUTION



In addition, most of disinfecting agents in MPDS are associated with **CYTOTOXIC EFFECTS** and induced varying levels of **IRREVERSIBLE TISSUE SENSITIVITY REACTIONS**.



Several MPDS showed a progressive **DOSE-DEPENDENT** increase in cytotoxicity for higher concentration.



This suggest that the formulations of MPDS need to asses the **AMOEBICIDAL EFFECTS** and **CYTOTOXICITY** and **BALANCE AMOEBICIDAL EFFECTIVENESS WITH LOW CYTOTOXICITY**.

Contact lens Solution	Active ingredient(s)	Other ingredients
Alcon Opti-Clean II	PolyQuad (0.001%)	Tween 21, MicroClens, edetate disodium (0.1%)
Alcon Opti-Free Express	PolyQuad (0.001%), Aldox (0.0005%)	Sodium citrate, sodium chloride, boric acid, sorbitol, AMP-95, Tetronic 1304, edetate disodium (0.05%)
Alcon Opti-Free RepleniSH	Propylene glycol, PolyQuad (0.001%), Aldox (0.0005%)	Sodium citrate, sodium chloride, sodium borate, TearGlyde, Tetronic 1304, nonannoyl ethylenediaminetriacetic acid
AMO Complete MoisturePlus	Polyhexamethylene biguanide (0.0001%), Poloxamer 237	Hydroxypropyl methylcellulose, propylene glycol, phosphate, taurine, edetate disodium, sodium chloride, potassium chloride, water
AMO UltraCare <sup>a</sup>	Hydrogen peroxide (3%)	Sodium stannate, sodium nitrate; buffered with phosphates and water
Bausch & Lomb Boston Simplus	Chlorhexidine gluconate (0.003%), polyaminopropyl biguanide (0.0005%)	Poloxamine, hydroxyalkylphosphonate, boric acid, sodium borate, sodium chloride, hydroxypropylmethyl cellulose, Glucam
Bausch & Lomb ReNu MoistureLoc	Alexidine (0.00045%)	Boric acid, sodium chloride, sodium phosphate, hydranate, poloxamine, MoistureLoc
Bausch & Lomb ReNu MultiPlus	Dymed (polyaminopropyl biguanide; 0.0001%)	Hydranate, boric acid, edetate disodium, poloxamine, sodium borate, sodium chloride
Ciba Vision Clear Care <sup>a</sup>	Hydrogen peroxide (3%)	Sodium chloride (0.79%), phosphonic acid, phosphate-buffered system, Pluronic 17R4
Ciba Vision AQUify	Polyhexanide (0.0001%)	Sorbitol, tromethamine, pluronic F127, sodium phosphate, dihydrogen, dexpanthenol, edetate disodium dehydrate
Kirkland Signature Multipurpose Solution	Polyaminopropyl biguanide (0.0001%)	Poloxamer 237, edetate disodium, sodium chloride, potassium chloride, water

<sup>a</sup> Hydrogen peroxide-containing solution.

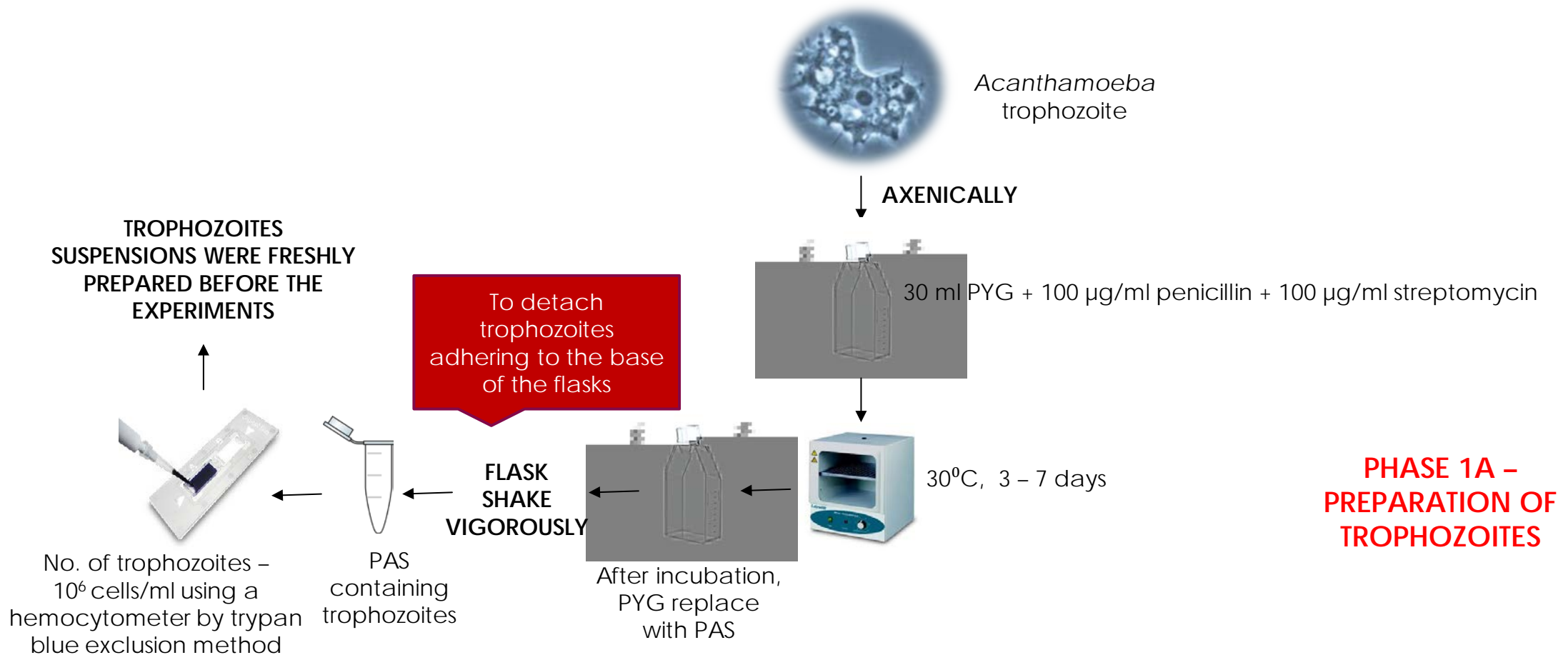
Table 4. Contact lens solutions tested and their ingredients.





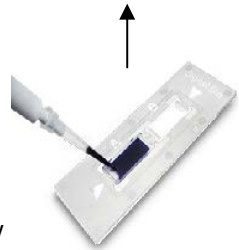
Figure 10. MPDS sold in Malaysia that claimed effective towards killing *Acanthamoeba*.

# MPDS EFFECTIVENESS TESTING METHODS

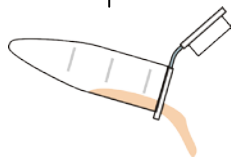


# MPDS EFFECTIVENESS TESTING METHODS

**CYSTS SUSPENSIONS WERE STORED AT 4°C AND USED WITHIN 2 DAYS**



Viable cysts no. –  $10^6$  cells/ml using a hemocytometer by trypan blue exclusion method



Supernatants were discarded and pellets were washed twice by re-suspension in PAS



3000 rpm, 10 min



After incubation, cysts transferred to sterile tube

They were already detached from the flask surface

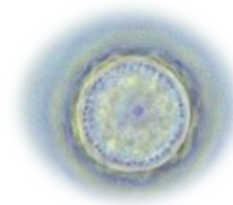


30 ml PYG + 100 µg/ml penicillin + 100 µg/ml streptomycin



30°C, 3 – 7 days

*Acanthamoeba* mature cysts were obtained from cultured aged in flasks for up to 7 days at 30°C.



**PHASE 1B – PREPARATION OF CYSTS**

# MPDS EFFECTIVENESS TESTING METHODS

The list of examined contact lens care solutions.

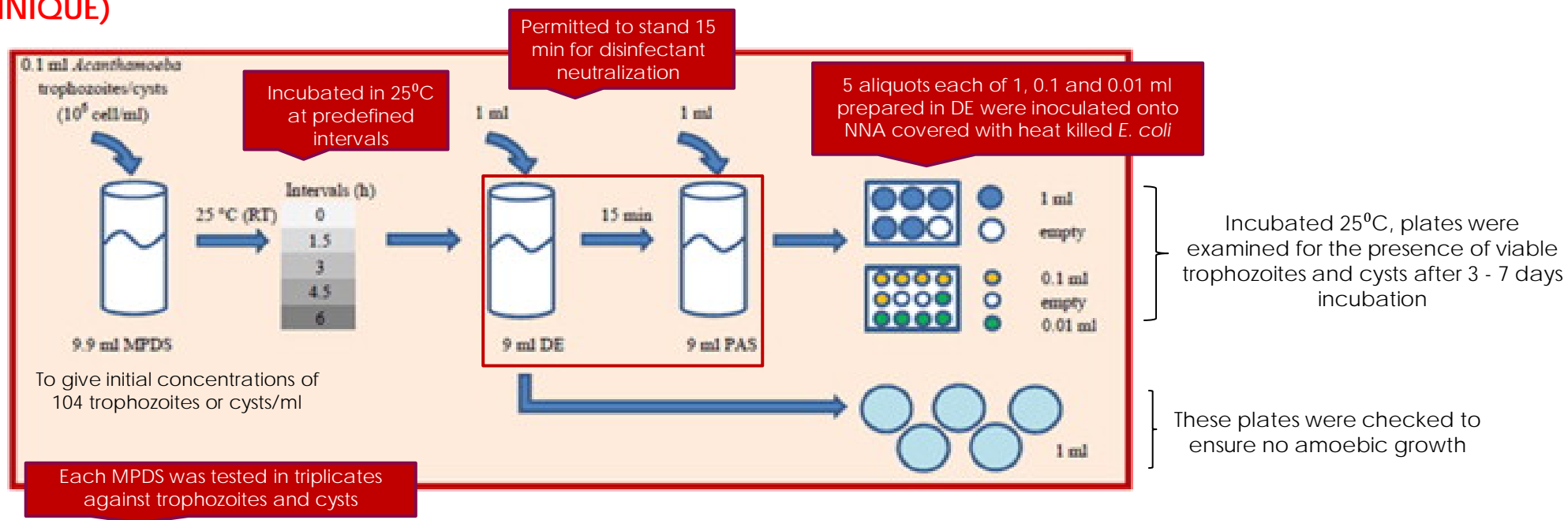
Solution	Active compound	Minimum disinfection time* (h)
ReNu MultiPlus	0.0001% polyaminopropyl biguanide	4
Opti-Free, Solo Care Aqua	Propylene glycol	6
AoSept Plus**	0.0001% polyhexanide 3% hydrogen peroxide	4 6

\* According to the minimum disinfection time recommended by manufacturers.

\*\* One-step system, with the platinum coated disc.

# MPDS EFFECTIVENESS TESTING METHODS

## PHASE 3 – ACTIVITY ASSAYS (MOST PROBABLE ENUMERATION TECHNIQUE)



The average log reductions were calculated using the MPN trophozoites and cysts per ml of MPDS at the designated time points.

$$\text{Log (viable cell at 0 h)} - \text{log (viable count at each interval time)}$$

# MPDS EFFECTIVENESS TESTING METHODS

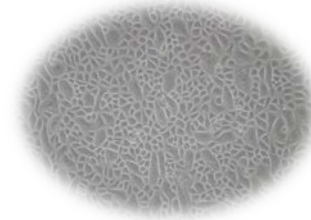
## PHASE 4 – CYTOPATHIC EFFECTS ON HUMAN CORNEAL EPITHELIAL CELLS

Percent CPE was calculated according to the following formula

$$\% \text{ CPE} = 100 - [(\text{OD of experimental well} - \text{OD of HCE alone}) / \text{OD of control cells}] \times 100$$

\*CPE assay was performed in triplicate

Cytopathic effects were assessed visually after Giemsa staining and measurement of optical density at 590 nm, with 0.1 ml of cells solubilized in 5% SDS.

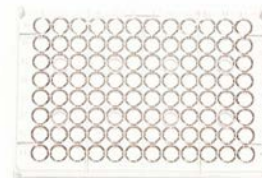


Human corneal epithelial cells

CULTURED



Endothelial cell growth media kits, in 5% CO<sub>2</sub> incubator



Monolayered HCE cells were placed in 96-well plates and incubated with selected MPDS or control media in 5% CO<sub>2</sub>

# MPDS EFFECTIVENESS TESTING RESULTS

Average<sup>a</sup> log<sub>10</sub> counts of *A. castellanii* ATCC 50373 trophozoites and cysts at the start of experiment (0 h) and after each predefined intervals (1.5, 3, 4.5 and 6 h), and average log reductions for trophozoites and cysts after each predefined intervals (1.5, 3, 4.5 and 6 h).

Time (h)	Stage	Control (PAS)		OPTI-FREE® PureMoist®	
		Avg. (SD) <sup>d</sup> log <sub>10</sub> counts	Log reductions	Avg. (SD) log <sub>10</sub> counts	Log reductions
0	Troph <sup>b</sup>	3.43 (0.08)		3.55 (0.14)	
	Cyst	4.04 (0.12)		4.12 (0.12)	
1.5	Troph	3.76 (0.31)	-0.33	<1 (0)	~3.55
	Cyst	4.16 (0.14)	-0.12	3.38 (0.13)	0.74
3	Troph	3.61 (0.09)	-0.18	0 (0)	3.55 (TK <sup>c</sup> )
	Cyst	4.06 (0.14)	-0.02	3.41 (0.25)	0.71
4.5	Troph	3.88 (0.11)	-0.45	0 (0)	3.55 (TK)
	Cyst	3.96 (0)	0.08	3.11 (0.19)	1.01
6	Troph	4.04 (0.12)	-0.61	0 (0)	3.55 (TK)
	Cyst	3.88 (0.11)	0.16	3.37 (0.13)	0.75

<sup>a</sup> The average based on the average of three different MPDS lots.

<sup>b</sup> Trophozoites.

<sup>c</sup> Total kill.

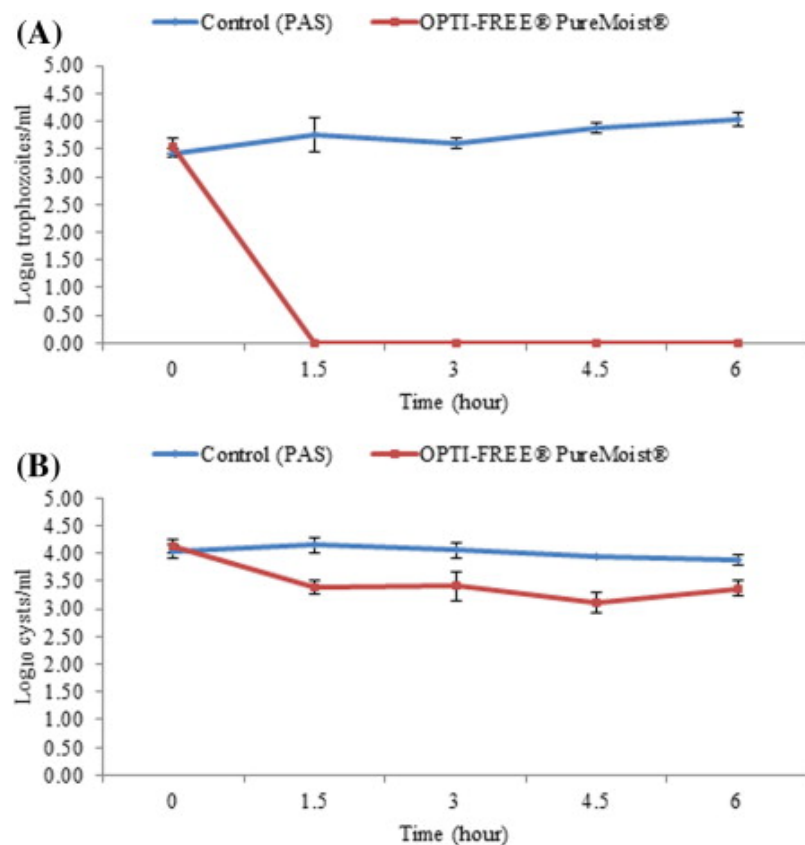
<sup>d</sup> Standard deviation.

CLs that achieved a 3-log reduction of amoeba during the MMRDT was considered as an effective disinfectant

# MPDS EFFECTIVENESS TESTING RESULTS

OPTI-FREE® PureMoist® achieved total kill against *A. castellanii* trophozoites by 3 h, 4.5 h and 6 h, respectively.

Cysts of *A. castellanii* were still viable after MMRDT (6 h).



Exposure time (h)	<i>A. castellanii</i> ATCC 50373	
	Troph <sup>a</sup>	Cyst
0	+	+
1.5	+	+
3	-	+
4.5	-	+
6	-	+

<sup>a</sup> Trophozoites.

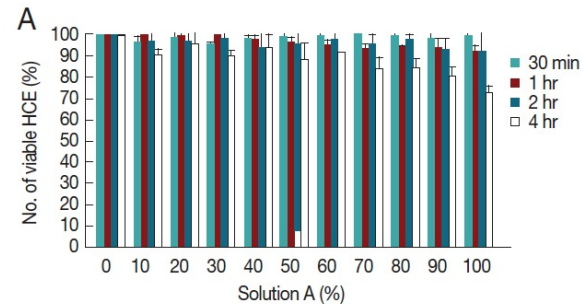
Table 5. Viability of *Acanthamoeba* strains after each time intervals.

Figure 11. Average trophozoiticidal (A) and cysticidal (B) effects of OPTI-FREE® PureMoist® over 6 h against *A. castellanii*.

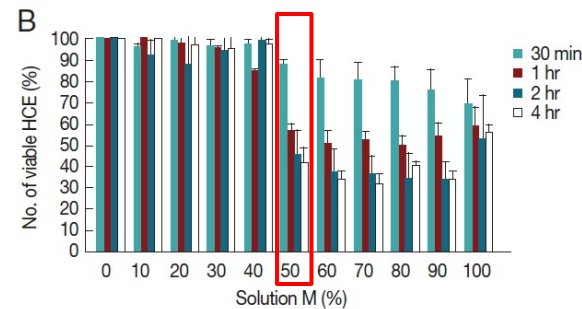


# MPDS EFFECTIVENESS TESTING RESULTS

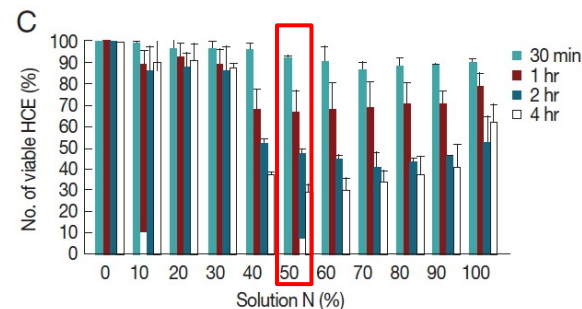
**LESS AMOEBCIDALS EFFECTIVE** – not cytotoxic at any of the concentrations tested.



**SOLUTIONS M & N SHOWED A TIME-DEPENDENT CYTOTOXICITY TOWARDS HCE CELLS. IT SHOWED THAT MPDS ALSO HAVE VARIOUS RANGES OF CYTOPATHIC EFFECTS AGAINST HUMAN CELLS.**



**MODERATE AMOEBCIDALS EFFECTIVE** – 43% cytopathic effects at 50% concentration at 1 h exposure.



**BEST AMOEBCIDALS EFFECTIVE** – 32% cytopathic effects at 50% concentration at 1 h exposure.

# PROBLEM OF ACANTHAMOEBA-TESTING FOR MPDS

“The ideal MPDS should maintain a **HIGH LEVEL OF DISINFECTION EFFICACY, PROVIDE SUPERIOR CLEANING** and **EXTENDED MOISTURE**, and be **STABLE FOR LONG-TERM STORAGE** avoiding toxicity to the eye”.



Food & Drug Administration (FDA) – minimum 1-log reduction of the initial inoculum was suggested as an appropriate measure of effective amoebicidal activity.



**JUST A SINGLE SURVIVING CYST CAN GIVE RISE TO A NEW ‘AMOEBIA POPULATION’**



Contents lists available at ScienceDirect

Contact Lens & Anterior Eye

journal homepage: [www.elsevier.com/locate/yexpr](http://www.elsevier.com/locate/yexpr)

Status of the effectiveness of contact lens solutions on keratitis-causing pathogens

Ruqaiyyah Siddiqui, Sahreena Lakhundi, Naveed Ahmad  
Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan

ORIGINAL ARTICLE

## Variable Responses of *Acanthamoeba* Strains to Three Multipurpose Lens Cleaning Solutions

MEGAN SHOFF, MS, ANDREW ROGERSON, PhD, SCOTT SCHATZ, PhD, OD,  
and DAVID SEAL, MD

*Oceanographic Center, Nova Southeastern University, Dania Beach, Florida (MS), College of Science, Marshall University, Huntington, West Virginia (AR), College of Optometry, Nova Southeastern University, Davie, Florida (SS), and Applied Vision Research Centre, City University, London, United Kingdom (DS)*

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solutions on *Acanthamoeba*



Magdalena Chruścińska<sup>a</sup>, Konrad Perkowski<sup>c</sup>,

<sup>a</sup>Warsaw, Poland  
<sup>b</sup>Warsaw, Poland  
<sup>c</sup>Warsaw, Poland

Jpn J Ophthalmol (2011) 55:547–557  
DOI 10.1007/s10384-011-0062-y

LABORATORY INVESTIGATION

ORIGINAL ARTICLE

## Efficacy of Korean Multipurpose Solutions against *Acanthamoeba*

Eun-Kyung Moon<sup>1</sup>, Hyeon-Young Kim<sup>1</sup>

<sup>1</sup>Department of Medical Zoology, Kyung Hee University School of Medicine, College of Medicine, Seoul, Korea

It's not very effective.

slak450

Vol. 47, No. 7

## Disinfection in Multiple *Acanthamoeba* Strains<sup>▽</sup>

Sharon Roy,<sup>1</sup> Jennifer Verani,<sup>1</sup> Michael J. Beach,<sup>1</sup> and Govinda Visvesvara<sup>1,2,3</sup>  
<sup>1</sup>Centers for Disease Control and Prevention, Division of Field Epidemiology, Atlanta, Georgia  
<sup>2</sup>and Epidemic Intelligence Service Program,<sup>3</sup> Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta, Georgia

## EFFICACY OF CONTACT LENS SOLUTIONS ON THAI CLINICAL ISOLATED *Acanthamoeba*

Darawan Wanachiwanawin<sup>1</sup>, Panida Kiataramkulchai<sup>1</sup>, Lalita Siridumrong<sup>1</sup> and Jeronimo A. S. Silva<sup>2</sup>

<sup>1</sup>Department of Parasitology, <sup>2</sup>Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahavithayalai, Bangkok, Thailand

International Journal of Life Sciences ISSN: 2231-6345 (Online)  
International Journal Available at <http://www.cibtech.org/jls.htm>  
April-June, pp.95-100/Paul et al.  
Research Article

## EFFECT OF CONTACT LENS SOLUTIONS ON *ACANTHAMOEBA POLYPHAGA* RESPONSIBLE FOR EYE DISEASE AMOEBIC KERATITIS

\*Newton Paul<sup>1</sup>, Tabrez Ahmad<sup>2</sup> and Sharma A.K.<sup>2</sup>

<sup>1</sup>Department of Zoology, Isabella Thoburn College, Lucknow

<sup>2</sup>Department of Zoology, Protozoology Research Laboratory, University of Lucknow, Lucknow, 226007, U.P, India

\*Author for Correspondence



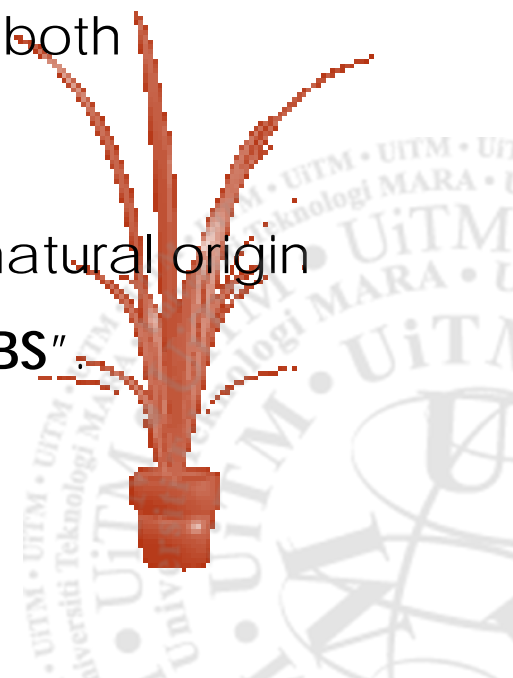
## *Acanthamoeba* cleaning solutions

Caroline de Oliveira Silveira<sup>1</sup>, Marilise Brittes Rott<sup>1\*</sup>

<sup>1</sup>Instituto de Física de Caruaru, Universidade Federal de Pernambuco, Instituto de Ciências Básicas da Saúde, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil

“The reported and worrying lack of effective cleaning solutions have urged researchers in this field to search for **NOVEL COMPOUNDS** as a high priority to be included as part of a new class of MPDS that are susceptible of both *Acanthamoeba* cysts and trophozoites”.

“Thus, there is a raising trend to shift resources from chemical drugs to natural origin compounds, mainly isolated from **MEDICINAL PLANTS** and **HERBS**”.



Plant	Extract	Effective Concentration (trophozoite)	Effect time (trophozoite)	Percentages of viable trophozoites	Effective Concentration (cyst)	Effective time (cyst)	Percentages of viable cysts
<i>Thymus silyoleus subsp. Silyoleus var. Silyoleus</i>	Methanol	32 mg/mL	3 h	0	32 mg/mL	12 h	0
<i>Satureja cuneifolia</i>	Methanol	32 mg/mL	24 h	0	32 mg/mL	72 h	53/07
<i>Melissa officinalis</i>	Methanol	32 mg/mL	72 h	55/07	32 mg/mL	72 h	70/0
<i>Trigonella foenum graecum</i>	Chloroformic	10 mg/mL	48 h	0	10 mg/mL	72 h	0
<i>Origanum syriacum</i>	Methanol	32 mg/mL	3 h	0	32 mg/mL	24 h	0
<i>Helianthemum lippii</i>	Ethyl acetate	N/A*	N/A*	N/A*	500 mg/mL	72 h	25
<i>Arachis hypogaea L</i>	Ethanol	N/A*	N/A*	N/A*	100 mg/mL (MIC**)	24 h	0
<i>Curcuma longa L</i>	Ethanol	N/A*	N/A*	N/A*	1 g/ml (MIC)	48 h	0
<i>Pancreatum maritimum L</i>	Ethanol	N/A*	N/A*	N/A*	200 mg/mL (MIC)	72 h	0
<i>Inula oculus-christi (L)</i>	Aqueous	32 mg/mL	24 h	0	32 mg/mL	72 h	74/7

\*N/A= Not Applicable

\*\*MIC= Minimum Inhibitory Concentration.

Table 6. Several medicinal plants with reported activity against *Acanthamoeba* cysts and trophozoites.

# MEDICINAL PLANTS AS A SOURCE OF NOVEL THERAPEUTIC COMPOUNDS



Figure 12. *Thymus sipyleus* subsp. *Sipyleus* var. *sipyleus*

- ① *In-vitro* effect of methanolic extracts of *Thymus sipyleus* subsp. *Sipyleus* var. *sipyleus* was tested against *Acanthamoeba* trophozoites (1.0 – 32 mg/mL).
- ② The effective activity was observed at 32 mg/mL. This medicinal plant presented no toxicity to human keratocytes even at the highest concentration tested (32 mg/mL).
- ③ A bio-guided fractionation analysis of *Thymus sipyleus* could help to find the active compounds within this plant against *Acanthamoeba* in the near future.

# MEDICINAL PLANTS AS A SOURCE OF NOVEL THERAPEUTIC COMPOUNDS



Figure 13. *Trigonella foenum graecum*

- ① The aqueous extract of *Trigonella foenum graecum* is active against both the trophozoite and cyst stages of *Acanthamoeba*.
- ② The concentration of 10 mg/mL was able to eliminate trophozoites and cysts when incubated at a concentration of 750 mg/mL after 48 h (trophozoite) and 72 h (cysts).
- ③ *Trigonella foenum graecum* did not shown toxicity when tested on cell culture at the highest evaluated concentrations.

# MEDICINAL PLANTS AS A SOURCE OF NOVEL THERAPEUTIC COMPOUNDS



Figure 14. *Origanum syriacum*

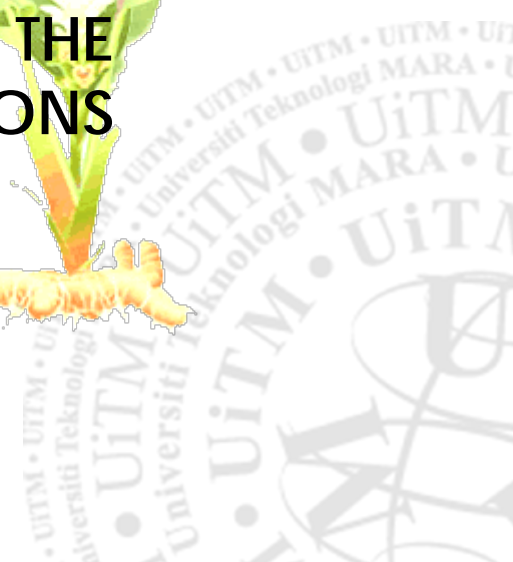
- ① *In-vitro* evaluation of the amoebicidal activity of methanolic extracts of *Origanum syriacum* against *Acanthamoeba castellanii* have shown that concentrations of 32 mg/mL was able to eliminate trophozoites after 3 h.
- ② Incubation of cysts with extracts at the same concentration (32 mg/mL) revealed a cysticidal activity after 24 h.



# MY RESEARCH FOCUS

NEW

**IN-VITRO AMOEBICIDAL ACTIVITY OF *CURCUMA LONGA* LINN.  
AGAINST *ACANTHAMOEBA* GENOTYPE T4 AND THEIR CYTOTOXIC  
POTENTIALS ON HUMAN CORNEAL EPITHELIAL CELLS FOR THE  
IMPROVEMENT OF CONTACT LENS DISINFECTING SOLUTIONS**



# THE SELECTION OF THIS PLANT WAS MADE ON THE BASIS OF INFORMATION GATHERED ABOUT IT USE IN THE LOCAL TRADITIONAL MEDICINE

Anti-  
protozoal

Anti-  
inflammatory

Anti-  
bacterial

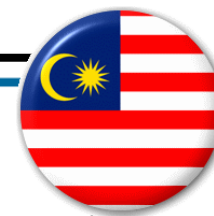
## A comprehensive review on *Curcuma longa* Linn.: Phytochemical, pharmacological, and molecular study

Kamran Ashraf<sup>1,2</sup>, Sadia Sultan<sup>1,2</sup>

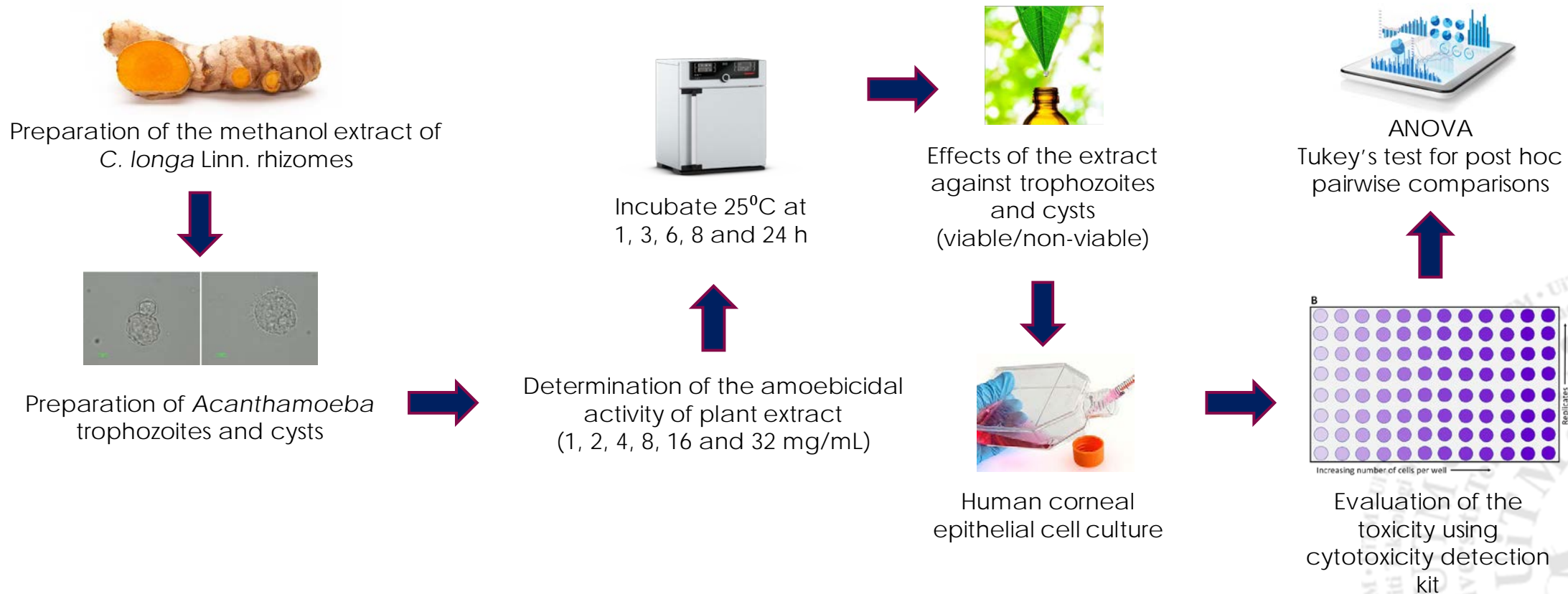
<sup>1</sup>Department Pharmacology and Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia, <sup>2</sup>Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia

### Abstract

*Curcuma longa* Linn. is well-known and valued medicinal plant. It has a long history of traditional uses ranging from folk medicine to several culinary preparations. The phytochemical, pharmacological, and molecular studies of *C. longa* are reviewed. The rhizome is rich in essential oils, and various numbers of chemical constituents with biomedical significance have been isolated from it. The management of indigenous knowledge by appropriate documentation is recommended. This review was compiled to provide recent consolidated information covering different aspects of the plant, phytochemical, pharmacological, and molecular study to provide a basis on which to plan future studies and to promote sustainable use of *C. longa*.



# EXPERIMENTAL DESIGN



# EXPECTED OUTCOMES

Dose (mg/ml)	Effect on	Experimental periods				
		1 h	3 h	6 h	8 h	24 h
32.0	Trophozoites					
	Cysts					
16.0	Trophozoites					
	Cysts					
8.0	Trophozoites					
	Cysts					
4.0	Trophozoites					
	Cysts					
2.0	Trophozoites					
	Cysts					
1.0	Trophozoites					
	Cysts					
Control	Trophozoites					
	Cysts					

Data were expressed as mean  $\pm$  SD

Table 7. Effect of *C. longa* Linn. methanol extract on the proliferation of *Acanthamoeba* genotype T4 trophozoites and cysts.

<i>Curcuma longa</i> Linn. (mg/mL)	% Cell viability
32.0	
16.0	
8.0	
4.0	
2.0	
1.0	
Control	

Table 8. Cytotoxic effect of *C. longa* Linn. on corneal cells using MTT.

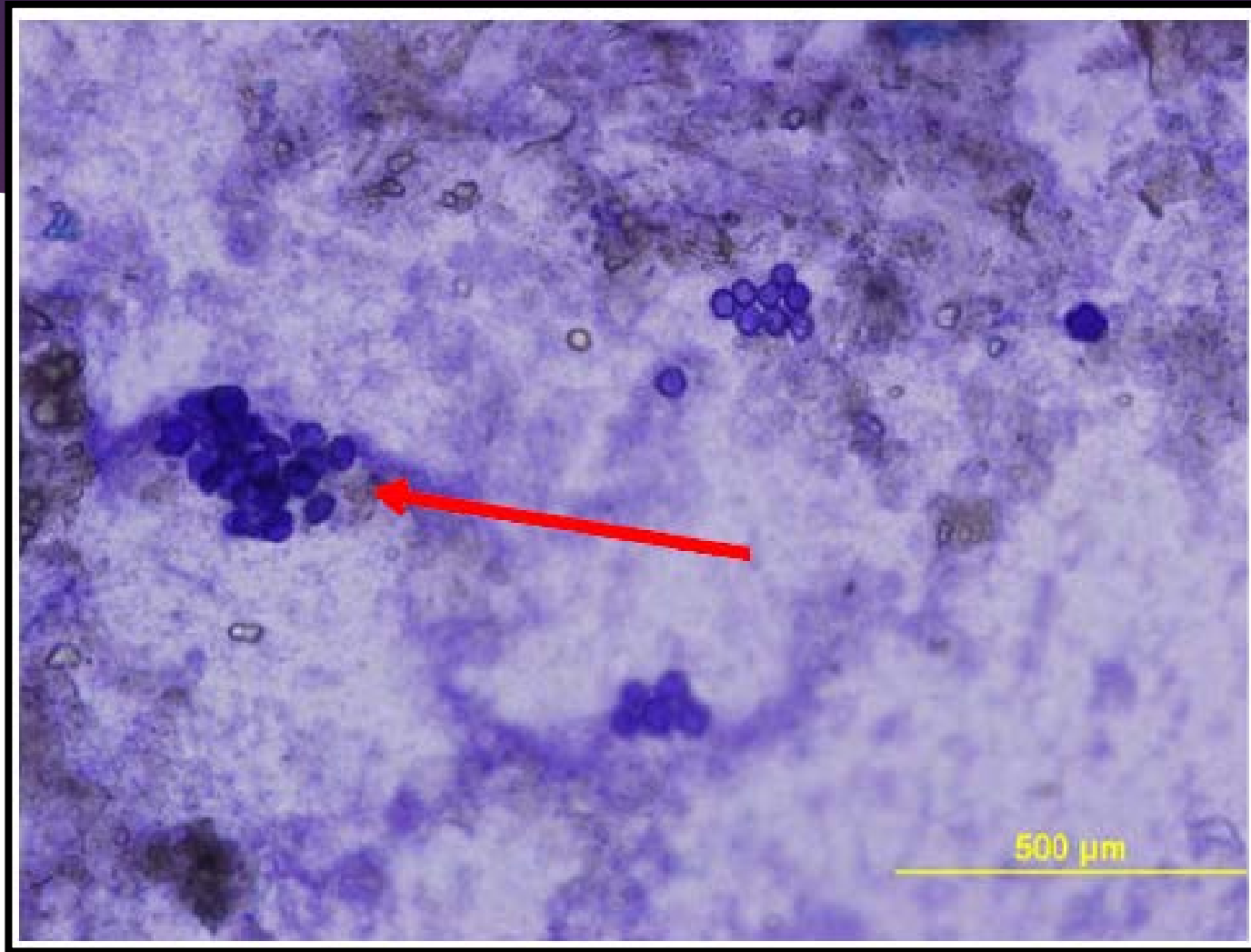


Figure 15. *Acanthamoeba* genotype T4 cysts treated with 32.0 mg/mL of *C. longa* Linn. extract at the 24 (optical microscope magnification x400). Dead cysts that are **dark blue** stained with trypan blue shown on the **tip of the arrow**.

## In vitro amoebicidal activities of *Satureja cuneifolia* and *Melissa officinalis* on *Acanthamoeba castellanii* cysts and trophozoites

E. Malatyali • B. Tepe • S. Degerli • S. Berk

### In-vitro Activity of *Trigonella foeniculum* as a Clinical Strain of *Acanthamoeba castellanii*

Samira Dodangeh<sup>a,b</sup>, Maryam Niyiyati<sup>a,b,c\*</sup>, Mohar  
Abdolali Moshfe<sup>f</sup>, Ali Haghig

## Screening of the in vitro amoebicidal activities of *Fusumaca armenea* (Fisch. & C.A.Mey.) and *Inula oculus-christi* (L.) on *Acanthamoeba castellanii* cysts and trophozoites

Serpil Degerli • Seyda Berk • Erdogan Malatyali • Bektas Tepe

**MANY MEDICINAL PLANTS/HERBS HAVE BEEN REPORTED TO PRESENT HIGH ANTI-ACANTHAMOEBA ACTIVITIES IN THE RECENT YEARS. THEREFORE, PLANTS EXTRACT SHOULD BE CONSIDERED AS A HIGHLY IMPORTANT AND POWERFUL SOURCE FOR THE SEARCH OF NOVEL ANTI-ACANTHAMOEBA COMPOUNDS IN THE NEAR FUTURE...**

## Bioassay guided isolation and identification of anti-*Acanthamoeba* compounds from Tunisian olive leaf extracts

Ines Sifaoui<sup>a,\*</sup>, Atteneri López-Arencibia<sup>b,2</sup>, Juan Carlos Ticona<sup>c</sup>, Carmen M<sup>a</sup> Martín-Navarro<sup>b,d,2</sup>, María Reyes-Batlle<sup>b,2</sup>, Mondher Mejri<sup>a,1</sup>, Jacob Lorenzo-Morales<sup>b,2</sup>, Antonio Ignacio Jiménez<sup>c</sup>, Manef Abderabba<sup>a,1</sup>, José E. Piñero<sup>b,2</sup>

## In vitro Amoebicidal Activity of Garlic Extract on *Acanthamoeba castellanii* and its Potential on Corneal Cells

SE VURAL,<sup>2</sup> FATIH OZAN,<sup>3</sup> BEKTAS TEPE,<sup>4</sup> CELIK,<sup>5</sup> and ALI CETIN<sup>6</sup>

## Investigation of in vitro amoebicidal activities of *Ornithogalum sigmoideum* on *Acanthamoeba castellanii* cysts and trophozoites

Bulent Kaynak<sup>1</sup>, Zeynep Koloren<sup>1</sup>, Ulku Karaman<sup>2</sup>

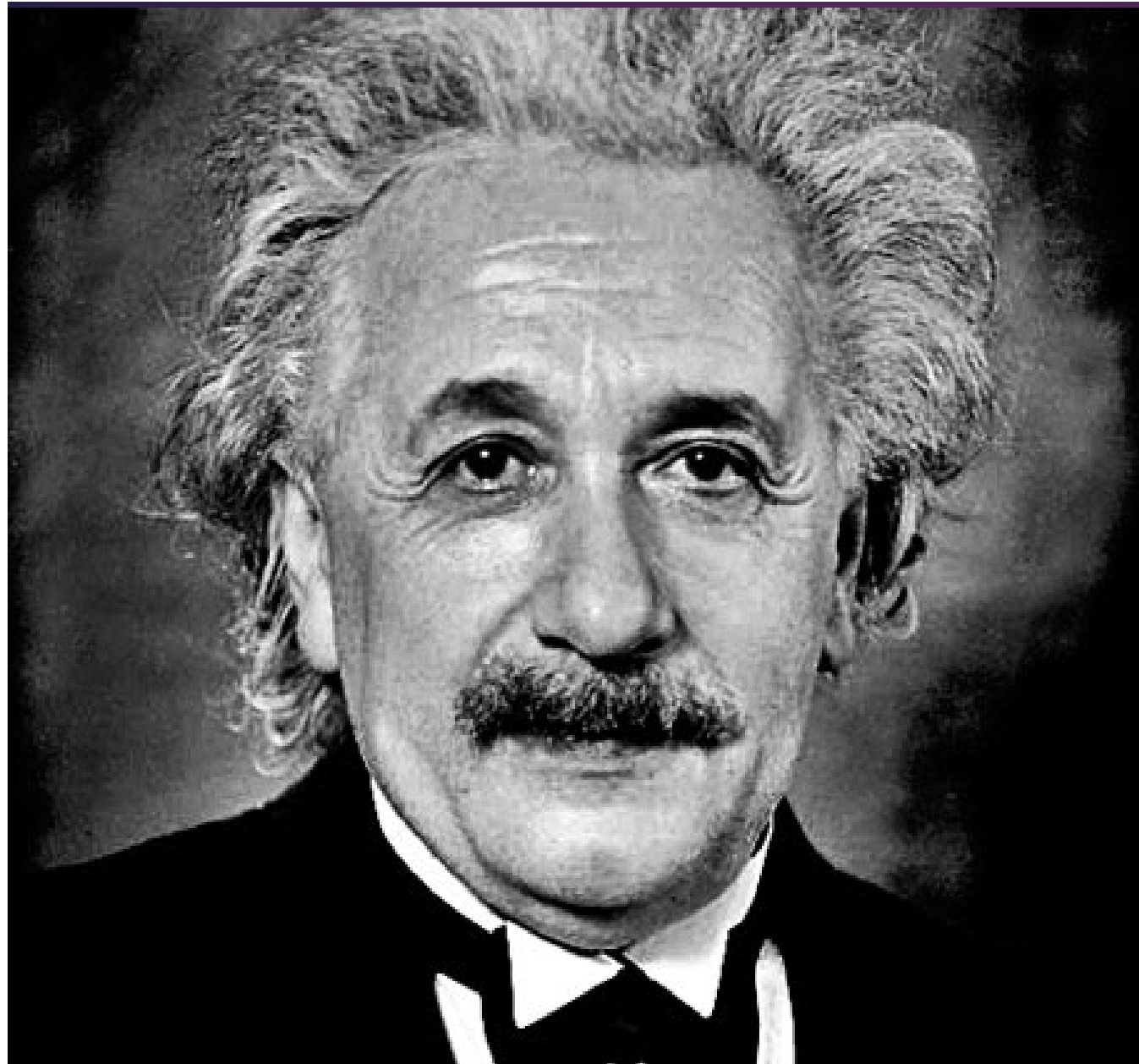
## SOMETHING TO PONDER...

“The number of contact lens wearers is estimated at 125 million in 2010. The contact lens market is estimated at **\$6.1 billion** in 2012, and it is estimated that the global market reached **\$11.7 billion** by 2017”.

“For a multibillion-dollar industry, it is puzzling that pharmaceutical companies are not investing in this research, especially a novel molecules/inhibitors/drugs and their clinical applications can be patented, which offer tremendous commercial value”.







“If we knew what it was we were doing, it would not be called research, would it?”

**Albert Einstein**

# ACKNOWLEDGEMENTS



**SUNWAY**  
UNIVERSITY



Prof. Dr. Naveed Ahmed Khan



Dr. Salleh Mohd Rofiee



Prof. Dr. Ruqaiyyah Siddiqui



اَوْبُو سَيِّدِي نَبِيكُو لَو كِنِي مَنَا رَا  
**UNIVERSITI**  
**TEKNOLOGI**  
**MARA**



Prof. Dr. Mohamed Kamel Abd Ghani



Rosnani Hanim Mohd Hussain



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*The National University*  
*of Malaysia*

*Thank  
You*

