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Free-living Amoebae (*Naegleria fowleri* and *Acanthamoeba*)

INTRODUCTION

Free-living amoebae are protozoans with complex life-cycles that occur naturally in aquatic and soil environments where they play an important ecological role. Their presence is not related to human or animal faecal contamination. In Australia there are two groups of free-living amoebae that have been associated with opportunistic infections in humans, *Naegleria* and *Acanthamoeba*. **ALS Water Melbourne and Perth laboratories now offer NATA accredited analysis of amoebae in waters including PCR confirmation (Melbourne).**

Figure 1: *Acanthamoeba* observed under the microscope feeding on bacteria.

METHOD INFORMATION

ALS METHOD CODE
MP684 – Identification of amoebae
MP688 – PCR confirmation of *Naegleria* spp., *Naegleria fowleri* and or *Acanthamoeba* spp.

LIMIT OF DETECTION
1 Amoeba per 500 mL

GUIDELINE LIMIT
Thermophilic *Naegleria* spp. should not be detected in a 500 mL sample of drinking or supply water.

NAEGLERIA FOWLERI

Naegleria fowleri is the most notorious free-living amoeba owing to its etiology with the rare but fatal disease PAM (primary amoebic meningoencephalitis). Infection occurs via intranasal pathways rather than ingestion therefore the greatest risk of infection is through recreational bathing. Chlorine is effective in treating contaminated water supplies although chloramination is required to ensure adequate disinfection of entire water distribution systems. *N. fowleri* is a thermophilic amoeba and has been detected in every Australian state and territory. Growth is fastest above 42ºC, but it also occurs at temperatures as low as 25ºC. Below 18ºC it is thought to occur as a dormant cyst. Although there are a number of species of *Naegleria* in Australian waters not associated with disease, the presence of any thermophilic amoeba is usually of concern.

ACANTHAMOEBA

Acanthamoeba spp. occur naturally in marine, freshwater and soil and are the most commonly isolated amoeba from environmental waters. Several species are known to infect humans causing granulomatous amoebic encephalitis and/or amoebic keratitis. Amoebic keratitis is a severe infection of the cornea and clinical cases have been reported in all Australian states although the source of the infection is difficult to trace owing to the ubiquitous distribution of the free-living amoeba. Outdoor workers, immune-compromised patients, and those who wear contact lenses are most at risk. *Acanthamoeba* spp. Cysts are highly resistant to chlorine and therefore treatment of contaminated waters is difficult. *Acanthamoeba* spp. have been identified as carriers of other pathogenic bacteria including *Legionella* spp., *Listeria monocytogenes*, *Mycobacterium avian*, *Helicobacter pylori* and *E. coli*. The detection of *Acanthamoeba* spp. in cooling-tower water is an indication that *Legionella* spp. may be present.

ANALYSIS OF AMOEBAE

Water samples are concentrated and amoebae are detected by laboratory culture using bacteria as the food source. The identity of the amoebae as either *Naegleria fowleri Naegleria* spp. or *Acanthamoeba* spp. is confirmed by PCR analysis. PCR detects a specific DNA sequence of the target amoeba.

MONITORING REQUIREMENTS

The presence of any thermophilic *Naegleria* in a water sample indicates that the conditions are suitable for the growth *N. fowleri*. The Australian Drinking Water Guidelines (2015) require that remedial action is taken immediately if thermophilic *Naegleria* spp. are detected in drinking water and before the results of the PCR analysis for *N. fowleri* are known. The ADWG has specified a detection limit of 1 thermophilic *Naegleria* spp. in a 500 mL sample as an appropriate threshold for action.

Waters that favour the growth of thermophilic amoeba are typically monitored routinely. These include:

- All waters that maintain a temperature above 25ºC and/or periodically exceed 30ºC, *e.g.* tropical waters, thermal springs, ground water, heated swimming pools, spas and industrial cooling water.
- Piped water supplies that are above ground and/or do not use chloramination to disinfect the entire distribution system.
- Other non-domestic uses of water where there is a higher risk of infection, *e.g.*, contact-lens wash solutions, industrial eye-wash stations, hospital water and renal dialysis.

WEST AUSTRALIA GUIDELINES

The West Australia Department of Health requires routine monitoring of thermophilic amoeba during the months of the year when water temperatures within the distribution systems are likely to exceed 20ºC. Analysis must be performed by a NATA accredited laboratory and samples for the analysis of thermophilic amoeba are collected at the same time and place as bacteriological samples are collected. The Department of Health must be notified within one working day of any detection of thermophilic *Naegleria* spp. in a drinking water supply.

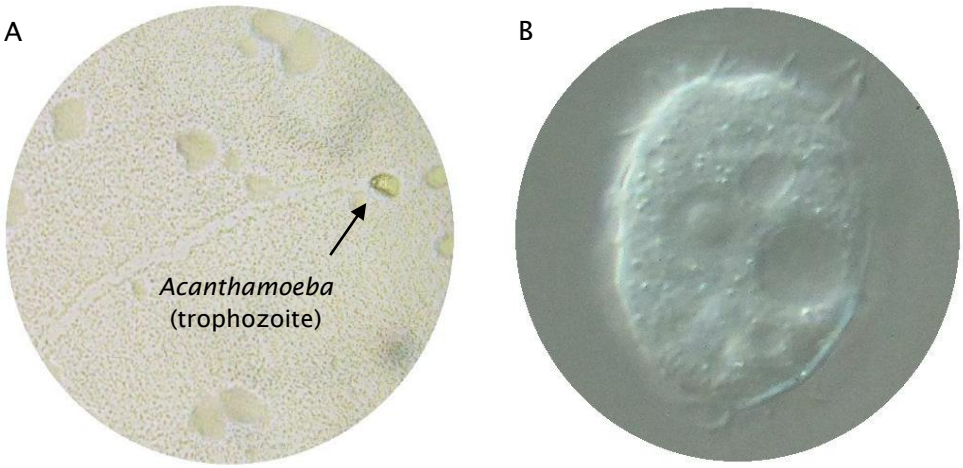

Figure 2: (A) *Acanthamoeba* observed under the microscope feeding on bacteria. The feeding stage of the amoeba life-cycle is known as the trophozoite. The track where the amoeba has ingested bacteria is clearly visible. (B) Differential interference contrast microscope image of *Acanthamoeba castellani* under high power magnification.

SAMPLING AND HOLDING TIME REQUIREMENTS

Holding Time	24 hrs (recommended) and up to 96 hrs (acceptable) as per AS2031:2012
Bottle Type	Sterile 500ml plastic bottle or pair of 250mL plastic bottles
Preservative	Sodium thiosulphate for chlorinated supplies only
Shipping and Storage	Transport and store at **ambient temperature** i.e. not in an esky. Samples should be bubble wrapped and sent in a cardboard box, wine box or express satchel

For further information please contact your local ALS Water or Environmental client services team.

REFERENCES

Visvesvara, G. S. (2010). Parasite Culture: Acanthamoeba *and* Naegleria *spp. In: Garcia LS, editor. "Clinical Microbiology Procedures Handbook. 3rd edition". Garcia, L. S. (ed.). ASM Press, Washington, DC.*

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