



Educational Introduction and Protocol for Molecular diagnosis of *Acanthamoeba* infection

Introduction:

Acanthamoeba spp. are a family of free-living protozoans ubiquitously distributed in the environment, commonly found in soil and fresh water. There are two stages to the *Acanthamoebae* life cycle: dormant cysts that survive in air, soil, dust and water; and a trophozoite stage that feeds on small algae, bacteria and other protozoa and (Dart et al 2009). Due to the ingestion of other microbes during the trophozoite stage, *Acanthamoeba* may act as a reservoir for human pathogens as endosymbionts.

Infection with *Acanthamoeba* spp. can cause cutaneous lesions and sinus infections, keratitis and granulomatous amoebic encephalitis. The latter is an amoeba infection of the central nervous system which is life threatening and often, but not always, seen in immunosuppressed patients.

Acanthamoeba encephalitis, first described in 1972 is an extremely rare infection of the central nervous system that is seen in both healthy and immunocompromised individuals. Primary amoebic meningoencephalitis (caused by *Naegleria fowleri*) and granulomatous amoebic encephalitis (caused by *Acanthamoeba* spp and *Balamuthia mandrillaris*) are the two clinical manifestations of CNS amoebic infection. Of the other 400 cases that have been reported in the literature, only two to three percent of those affected survived (Kaushal, 2008). Dissemination occurs when *Acanthamoebae* spread haematogenously from the upper respiratory tract or skin lesions into the brain parenchyma (Anderson et al, 2012).

Acanthamoeba meningoencephalitis is a slowly progressing infection with typical symptoms including headache, fever, neck stiffness, seizures, altered mental status and neurological symptoms leading to coma and death within one week to several months after onset. Treatment is difficult due to poor rates of diagnosis and a lack of antimicrobial therapy, resulting in the high mortality rate of the disease (Anderson et al, 2012). There are reports of successful treatment of patients using a combination of trimethoprim-sulfamethoxazole, rifampicin and ketoconazole (Singhal et al, 2001).

Amoebic keratitis, by comparison, is more common in the immunocompetent population and is associated with contact lenses use and poor lens hygiene. Corneal

trauma and contact with contaminated water also facilitate the transmission of the protozoan. Keratitis causes high morbidity and may result in permanent loss of vision.

In the UK, infection with *Acanthamoeba* is relatively rare but the consequences are life changing and keratitis is not easily treatable. Since keratitis is closely linked to contact lens usage the incidence of amoebic keratitis may be on the rise corresponding to the increase in contact lens wearers. Service users may wish to refer samples to us where keratitis aetiology is unknown.

Acanthamoebae have even made national news headlines in regard to the risk of infection associated with wearing contact lenses (<http://www.bbc.co.uk/news/health-32791627>). In the cornea *Acanthamoebae* are thought to feed on Keratocytes (Dart et al 2009). The pathogenicity is linked to their ability to cause host cell death by interfering with host cell signalling, secreting toxins and an ability to phagocytose host cells (Khan, 2006). The BBC article mentions 60 new cases of this particular infection.

In the same period at Micropathology Ltd. we detected 79 cases out of 741 specimens. This statistic whereby 10% of suspected specimens are positive, is in agreement with the French study of Maubon et al (2012). Common specimen types include corneal swabs, scrapes, contact lens fluid and contact lenses themselves.

At Micropathology Ltd we perform a probe based PCR assay for *Acanthamoeba* spp. detection based on molecular amplification with a two day target turnaround time.

In the case of *Acanthamoeba* keratitis, the far most common disease associated with *Acanthamoeba* infection, early detection followed by prompt treatment is essential for a good prognosis (Dart et al 2009). Traditional culture techniques involve inoculating the specimen onto a lawn of *Escherichia coli* on non-nutrient agar for 3-6 days with occasionally up to 3 weeks (Dart et al 2009; Maubon et al 2012). This is not in keeping with the urgency of the situation as the disease develops. Not only does PCR offer a more rapid diagnosis but it has also been suggested that detection by molecular amplification can also aid in the detection of extra-corneal spread of *Acanthamoeba* since accurate histological confirmation is often difficult (Dart et al 2009).

To aid in the production of a rapid service we have developed the following user protocol with our colleagues at Moorfields Eye Hospital in London.

User Protocol for Acanthamoeba DNA detection by molecular amplification:

Use a labelled universal container, containing a bijoux bottle, a ziploc poly bag, AND a request form.

- Complete a patient request form (attached below) with FULL history and details of what you require e.g. “3 weeks of keratitis, clinically *Acanthamoeba*, please exclude *Acanthamoeba*, fungus, bacteria, Herpes Simplex and Mycobacteria”.

Corneal specimens:

- Use a cotton swab with a plastic shaft and firmly swab the ulcer and epithelium to remove cells.
- Place the swab in a bijou bottle and **avoid contaminating the shaft**.
- Cut the shaft flush to top of bottle with sterile scissors and screw lid onto the bijou bottle.
- If the cornea is very thin it may be safer to use a Bard Parker D15 blade to take the specimen and transfer the cells to a plastic shaft cotton swab (**without contaminating the shaft**) and place this in the bijou bottle as above.
- Place the bijou bottle into a labelled Universal container and pack the container with tissue paper (to stop the vial rattling around resulting in loss of tissue from the swab).
- Put into the poly bag with the **completed request form** (<http://www.micropathology.com/customer-downloads-forms.php>).

Aqueous specimens:

- Use a 1ml syringe with a 30 g needle. *Micropathology* would like as much as possible. The average AC volume is only 175 µl try to send 100 µl or more.
- After removing from the eye withdraw the plunger to draw the aqueous into the syringe from the needle before removing needle and screwing a stopper onto the end.
- Put sellotape around the plunger to stop it emptying.
- Place into the poly path bag with a **completed request form** (<http://www.micropathology.com/customer-downloads-forms.php>).

Turnaround times

Turnaround times are stated in the user manual

(<http://www.micropathology.com/customer-downloads-handbook.php>) with results usually available in practice much sooner than the given time frame. Where there is a delay we are usually confirming a result and addressing clinical data given with the specimen. Results are given out by e-fax or email as interim or final reports, with a hard copy sent out by post once a week if required. We are also able to give results on request by phone.



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RAPID DIAGNOSIS OF EYE SAMPLES - Molecular testing (microbial DNA PCR identification)

Patient details OR Sticker:

Surname:

Forename(s):

DoB: (DD/MM/YYYY)

Gender: M / F / U

Hospital No:

Laboratory name and address for results:

Service & clinic code:

Consultant:

Requesting doctor:

Doctor phone/email:

Fax results to:

Sample type: e.g. Corneal Swab, Aqueous Tap

Date/Time taken:

Clinical details:

Site involved:

Date of onset of symptoms:

Results of previous tests:

Suspected organism(s):

Tests requested:

Bacteria

Acanthamoeba

Fungi

HSV

VZV

CMV

Please supply the contact details of your Finance department for invoicing.

References

Anderson, A., Ojano, S., Matela, I., Senya, C., Sovandy, C., and Vuthy, (2012) Acanthamoeba and multidrug-resistant *Escherichia coli* associated meningoencephalitis: A special case report. *Int J Med Biosci.* 1(4):69-72

Dart, J.K.G., Shaw, V. P. J. and Kilvington, S. (2009) Acanthamoeba Keratitis: Diagnosis and Treatment Update. *American Journal of Ophthalmology* **148**, 487–499

V Kaushal, DK Chhina, R Kumar, HS Pannu, HPS Dhoori, RS Chhina. (2008) Acanthamoeba encephalitis. *IJMM.* **26**:182-184

Khan, N. A. (2006) Acanthamoeba: biology and increasing importance in human health *FEMS Microbiology Reviews* **30**, 564-595

Maubon, D., Dubosson, M., Chiquet, C., Yera, H., Brenier-Pinchart, M-P, Cornet, M., Savy, O., Renard, R., Pelloux, H. (2012) A one-step multiplex PCR for acanthamoeba keratitis diagnosis and quality samples control. *Invest Ophthalmol Vis Sci.* **53**, 2866–2872

Singhal T, Bajpai A, Kalra V, Kabra SK, Samantaray JC, Satpathy G, Gupta AK. (2001). Successful treatment of Acanthamoeba meningitis with combined oral antimicrobials. *Pediatr Infect Dis J.* 20(6):623-627