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

Introduction

Acanthamoeba spp. are a family of free-living protozoans, that have two stages to their life cycle, a trophozoite stage that feeds on small algae, bacteria and other protozoa and dormant cysts that survive in air, soil, dust and water (Dart et al 2009).

At Micropathology Ltd we perform a test for Acanthamoeba sp. based on molecular amplification with a next day target turnaround time.

Acanthamoeba have recently made national news headlines as one of the perils of wearing contact lenses (http://www.bbc.co.uk/news/health-32791627). In the cornea Acanthamoebas 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 particularly infection. In the same period at Micropathology we detected 79 cases out of 741 specimens. At around 10% of suspected specimens being positive this 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.

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 over 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).

In the case of the far more common Acanthamoeba keratitis, for a good prognosis, early detection followed by prompt treatment is essential (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. 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 a user protocol with our colleagues at Moorfields Eye Hospital in London.

User Protocol for Acanthamoeba DNA detection by molecular amplification

Protocol:
Use a labelled universal container, containing a bijou 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

The turaround 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 meningo-encephalitis: 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

