Micropathology Ltd
Tel 24hrs: +44 (0) 24 - 76 – 323222
Fax / Ans: +44 (0) 24 - 76 - 323333
University of Warwick Science Park, Venture Centre, Sir William Lyons Road, Coventry CV4 7EZ
Website: www.micropathology.com E-mail: info@micropathology.com

Molecular diagnosis of *Acanthamoeba* infection

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 (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.

Most people will be exposed to this organism during their lifetime, but very few will become sick from this exposure as infection is rare. In these cases, the protozoa enter the eye typically through contact lens use or damage to the cornea, or enters the body through skin wounds or inhalation into the lungs with the potential to cause disease.

Disease caused by *Acanthamoeba*:

- **Acanthamoeba keratitis**
  - An infection of the eye that typically occurs in healthy persons and can result in permanent visual impairment or blindness
- **Disseminated infection**
  - A widespread infection which can affect the skin, sinuses, lungs, and other organs
  - More common in immunocompromised patients.
  - A further risk that organism will disseminate from the primary infection site to other areas such as the brain parenchyma (Anderson et al, 2012).
- **Granulomatous Amoebic Encephalitis (GAE)**
  - A serious and life-threatening infection of the brain and spinal cord with a high mortality rate (Kaushal, 2008).
  - Typically occurs in the immunocompromised/immunosuppressed

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 vison, therefore 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).

Service users may wish to refer samples to us where keratitis aetiology is unknown or where clinical history is indicative of *Acanthamoeba* spp. infection or where GAE meningitis is suspected. Accredited specimen types are CSF, corneal scrape, eye swab and contact lens fluid; other specimen types can be tested and reported along with an appropriate caveat stating that the assay is not UKAS accredited for testing of alternative sample types.

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.

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

Kaushal, V., China, DK., Kumar, R., Pannu, HS., Dhoori, HPS., China, RS., (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