

**O1204 Establishment of PCR for the early diagnosis of *Acanthamoeba* keratitis at Oslo University Hospital, Norway**

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**Background:** *Acanthamoeba* keratitis is a serious disorder, which may cause impaired vision and blindness. Early diagnosis and treatment is important for the prognosis. Detection is done in corneal tissue samples. The standard method has been cultivation, which is time consuming and has low sensitivity. Polymerase chain reaction (PCR) is therefore increasingly becoming the method of choice.

**Materials/methods:** A literature search for *Acanthamoeba* PCR protocols was performed. Published sets of primer and probe sequences were compared to GenBank DNA sequence entries with NCBI's nucleotide BLAST program. Sample DNA was extracted with the EZ1 DNA Tissue Kit on Qiagen EZ1. Samples containing *Acanthamoeba* trophozoites and cysts were used to determine optimal pre-extraction treatments; proteinase K, beads or both. Optimization of PCR conditions and repeatability and linearity of the optimized protocols were carried out using two ATCC strains (*A. castellani* and *A. polyphaga*). Clinical samples with *Acanthamoeba* sp., other relevant microbes and large amounts of human DNA were tested, and corneal tissue samples spiked with *Acanthamoeba* were examined for PCR inhibitors.

**Results:** Primers and probes from three publications (Schroeder *et al.* 2001, Rivière *et al.* 2006 and Qvarnstrom *et al.* 2006), all amplifying 18S rRNA gene regions, were selected for evaluation. The Schroeder *et al.* primers amplified human DNA and were excluded. The BLAST search showed that both the Rivière probe and reverse primer included one nucleotide mismatch compared to common *Acanthamoeba* GenBank sequences, while no polymorphism affected the Qvarnstrom PCR. After supplementing the Rivière protocol with reverse primer and probe and optimization, both PCR protocols showed good sensitivity for detecting *Acanthamoeba* sp., and good specificity in samples containing large amounts of human DNA or relevant microbes. Corneal tissue did not inhibit the PCR. Pre-extraction treatment with proteinase K gave the best combined DNA yield from trophozoites and cysts.

**Conclusions:** A PCR protocol with simultaneous testing using the Qvarnstrom PCR and the modified Rivière PCR in separate wells has now been implemented, with validation showing good sensitivity and specificity. We recommend that the PCR is performed on all corneal samples when there is question of *Acanthamoeba* keratitis.

