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Detection and identification of Acanthamoeba genotypes in corneal scrapings by real-time PCR and microbiome (16S/18S) analysis

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Background: *Acanthamoeba* is a free-living amoeba that can cause keratitis and lead to blindness if left untreated. The genus exhibits large genetic diversity, and to date, 20 genotypes (T1–T20) have been identified. Differential diagnosis of keratitis, which can also be caused by bacteria, fungi, and viruses, requires in-depth clinical expertise and is supported by microbiological diagnostics. A microbiome platform was developed for exhaustive detection and identification of ribosomal genes in clinical samples (16S/18S analysis), thus allowing detection of parasites, fungi, and bacteria. Due to sampling issues, high diagnostic sensitivity is essential to detecting *Acanthamoeba*-associated keratitis. In this study, we compared results obtained by state-of-the-art diagnostics (real-time PCR for *Acanthamoeba*) with total ribosomal gene (16S/18S) analysis in order to evaluate i) the sensitivity of 16S/18S analysis compared with real-time PCR, ii) the robustness of genotyping based on microbiome analysis, and iii) the clinical applicability of the microbiome platform in terms of simultaneous detection of fungi, parasites, and bacteria as causative agents of keratitis.

Material/methods: A total of 100 DNAs originally extracted from corneal scrapings and screened by *Acanthamoeba*-specific real-time PCR were randomly selected and submitted to microbiome profiling using the 16S/18S assay, involving amplicon-based PCR using general primers targeting prokaryotic and eukaryotic ribosomal genes and data analysis by the software BION and R statistics. Results from real-time PCR and microbiome profiling were compared. Consensus sequences from the BION output were queried in the NCBI Database for information on genotype and subject to phylogenetic analysis using reference sequences from the NCBI Database.

Results: Of the 100 DNAs, 19 and 14 were positive for *Acanthamoeba* by real-time PCR and 16S/18S analysis, respectively. All samples positive by the 16S/18S assay were positive by real-time PCR. The five samples negative by 16S/18S analysis but positive by real-time PCR had high cycle threshold values (range, 38–41), indicating low amounts of *Acanthamoeba*-specific DNA. The 16S/18S assay did not detect any cases not detected by real-time PCR. T4, T6, and T11 genotypes were found in the sample set (Table 1). Overall, there was fair agreement between the genotypes automatically called by BION and those identified by manual sequence analysis (BLAST and phylogeny). Fungal and bacterial species of potential clinical relevance identified in the sample set included *Staphylococcus aureus* (n=9), *Pseudomonas aeruginosa* (n=5), *Staphylococcus epidermidis* (n=24), *Streptococcus pneumoniae* (n=12), and *Fusarium solani* (n=6).

Conclusions: The 16S/18S platform was less sensitive than real-time PCR in terms of detecting *Acanthamoeba*-specific DNA in genomic DNA extracted from corneal scrapings. Meanwhile, robust information on genotype was provided by the 16S/18S assay and other pathogens of potential clinical relevance could be identified. Ongoing studies will reveal to which extent the assay will be applicable as a first-line diagnostic tool for infectious keratitis.