Reviewer's report

Title: Specific detection of fungal pathogens by 18S rRNA gene PCR in microbial keratitis

Version: 1 Date: 5 November 2007

Reviewer: Jorge Alio

Reviewer's report:

General

-----------------------------------------------

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Materials and methods section:

The methods section is badly structured. In the first place the Evaluation of the PCR method should be appear. And this subsection should be divided into “Strains”, DNA extraction (fungal, bacterial, human DNA) including the serial dilutions to test sensibility, Primers design, PCR assay and visualization of the product. And in the second place the analysis of the ocular samples, beginning with the Patient selection, sample collection, microscopy and culture, DNA extraction from ocular samples. PCR assay (both PCRs), DNA sequencing, and DNA analysis.

The ocular samples when the fungal presence was not proven for any test (stain, culture or PCR), should be excluded from the study. The percentages that appear in the text about number of samples where fungal presence has been detected in each technique can be interpreted as sensibility of the methodology used.

What is the final volume of the ocular sample when the DNA has been extracted?

Regarding the culture methodology, the temperature to grow the fungi in Sabouraud agar is 30°C not 37°C.

Figure 1. In the ITS2 region, there is not a primer binding region. Maybe the authors would like to show ITS/5.8S rRNA gene primers binding region.

Results section:

This section should be structured like the materials and methods section. In first place they should show the sensitivity and specificity of the new primers obtained in standard fungal strains. After that they should show the results obtained in ocular samples.

Epidemiology: The epidemiology is not the goal of the study. This paragraph should be removed.
PCR: The first sentence should be in material and methods. Rewrite this section without the samples whose fungal presence has not been proven. The results obtained by molecular methods in patients 2 and 9 seem to be due to PCR contamination, not culture contamination.

Discussion: This section should be rewritten excluding all the non-relevant data for the purpose of the study. In general this section is poorly written. The authors contradict themselves in their conclusions (page 12, line 13: “18S rRNA based PCR does not detect species of fungus”).

Conclusion: The authors even purpose other techniques (ITS amplification and sequencing) to identify the species.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
'I declare that I have no competing interests'