Reviewer's report

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

Version: 2 Date: 28 January 2008

Reviewer: Jorge Alio

Reviewer's report:

Main corrections.

There are many reports on the detection and identification of fungal pathogens in ocular infections. The authors show a new couple of primers to detect (without identification) the same as others authors describe but with the inconvenience that they are not able to identify the species by sequencing the amplified DNA with their primers (or they have not shown these results). Why did the authors not sequence the amplified DNA with the primers PFPRIM-F3 and PFPRIM-R4 to identify the species? If they have to use other primers to amplify and be able to identify the species, where are the advantages of their primers?

If the authors do not show clear advantages over other reports where not only the fungal detection but the identification is carried out, the manuscript is not publishable. The discussion is really poor. The goal of the study is not well defined (why do the authors test a new couple of primers if there are various primers described by other groups that work very well). They must discuss their results and compare them with other reports (for example: ITS1-ITS4-ITS86 results), showing the advantages versus other PCR detection methods.

Minor essential revisions:

Background:
- The authors must include more references about fungal DNA detection depending of the region of DNA amplified. Remove the acanthamoeba detection sentence.

Methods:
- page 5, first paragraph, first sentence: "Various filamentous fungi" versus "Various fungi"
- Table 2 appears in the text before table 1. Change the numeration.
- Page 6, last sentence. In the text it appears that the authors use only 1 pmol of primers, it is correct or it is a printing error?
- Page 8, 3rd paragraph, second sentence: "1000X and 400X" versus "400X and 1000X"

Results:
- page 10, second paragraph. The authors speak about 401 bp amplified DNA but in table 2, the size of the fragment of the reference strains is the 390±3bp. Which is the correct size?

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

**Level of interest**: An article of limited interest

**Quality of written English**: Acceptable

**Statistical review**: Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests**:

I declare that I have no competing interests