Reviewer's report

Title:A multiplex nested PCR for the detection and identification of Candida species in blood samples of critically ill paediatric patients

Version:1 Date:6 April 2014

Reviewer:Anna F Lau

Reviewer's report:

The manuscript by Taira et al describes the development of a multiplex nested PCR for the detection of seven Candida species in whole blood from critically ill pediatric patients. Results from 54 patients were compared with blood culture from simultaneous draws. Overall, the manuscript was well written and highlights the poor sensitivity of gold standard blood cultures.

Major comments:

1. Lines 174-177: Limit of detection was performed on C. albicans DNA only. It is well know that PCR efficiency differs between targets, particularly in multiplex reactions. The authors should provide limit of detection values for each of the seven targets within each respective multiplex assay.

2. Specificities of the assays were measured against bacteria and non-Candida DNA. It is unclear whether specificity testing was also performed among different Candida species. It is well know that Candida dubliniensis is genetically closely related to Candida albicans – is there potential for cross reaction? Were members of the C. parapsilosis complex (C. metapsilosis and C. orthopsilosis) tested for specificity of the C. parasilosis primers?

3. Figure 1 lane 7 is missing control bands for C. albicans and C. lusitaniae.

4. Figure 1 lane 14: the C. pelliculosa control band is very faint – does this indicate lack of PCR efficiency or low starting template concentration?

5. The authors find 3 patients with dual infection with C. parapsilosis and C. tropicalis. Further discussion in warranted. Is this pairing common and clinically significant? It is unusual that C. albicans DNA was not detected amongst the mixed infections given the higher proportion of disease caused by this species.

6. The authors should discuss their assay in the context of other new emerging technologies such as T2 biosystems, which address the poor sensitivity of blood culture for detecting candidemia using NMR.

7. Although the poor sensitivity of blood culture is well known, the authors do not address the potential of transient Candida DNAemia which can be a likely cause of false positives and unnecessary treatment.
Level of interest: An article of importance in its field

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.