Reviewer’s report

Title: A highly sensitive modified nested PCR to enhance case detection in leishmaniasis

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Reviewer: Srinivasan Ramakrishnan

Reviewer's report:

In this article the authors develop a modified nested PCR method for diagnosis of Leishmaniasis. Authors describe in detail the PCR strategy and primer design and test it against 70 patient samples (known 40 positives and 30 negatives). They compare the diagnostic efficacy of this method against light microscopy and in vitro culture and find it to better than these conventional methods. In conclusion the authors suggest that this method could be used to obtain better sensitivity and accuracy in diagnosis of L. donovani infections. Following are my concerns for this manuscript -

1) The authors mention that the inner primers are specific to Leishmania. Could the authors explain this better in the text. Is the region amplified by the inner primers absent in other kinetoplastids (leptomonas and crithidia)? Or are the primer annealing site sequence (and therefore the primer sequence) very unique in Leishmania when compared to other organisms? Such a difference can also be shown in figure 1 where a model diagram from other organisms can be included.

2) During the PCR, wouldn't the evaporation and condensation on the lid introduce the inner primers early in the PCR reaction? Did the authors use a negative control where tubes are not inverted after 1st round of amplification?

3) I notice that the extension time is same for both rounds of amplification. Shouldn't the larger band continue to be amplified in 2nd round of amplification also? Then it is surprising that there isn't a 603 bp band on the gel image in figure 2? Please explain.

4) The authors use a formula to determine the sample numbers for this study. However, it is not clear how the authors decided the input values such as accuracy, sensitivity prevalence etc. Did the authors consider previous studies for determining these values? If yes then please list references?

5) Authors provide reference for LM and IVC. However, I recommend that authors should also include a brief sentence or two explaining how many fields were examined for LM and how many days the authors waited before and IVC culture was marked negative?
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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Please indicate the quality of language in the manuscript:

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