



SDI Review Form 1.6

Journal Name:	British Microbiology Research Journal
Manuscript Number:	Ms_BMRJ_29557
Title of the Manuscript:	Evaluation of DNA based techniques for the diagnosis of Human Vaginal trichomoniasis in North Indian Population
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)



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PART 1: Review Comments

	Reviewer's comment The manuscript entitled "Evaluation of DNA Based Techniques for the Diagnosis of Human Vaginal Trichomoniasis in North Indian Population" addresses the diagnostic methods for human vaginal trichomoniasis based on <i>pfoB</i> gene PCR method compared with the conventional wet smear examination and 18S rRNA gene PCR methods. The manuscript will gain clarity from language editing by professionals. I have several comments, questions and suggestions that could help the authors to improve their manuscript:	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here). Corrections done as suggested by reviewer.
Compulsory REVISION comments	Abstract. Lines 28 and 29. The authors refer the efficacy of the three techniques: wet mount, 18S rRNA and <i>pfoB</i> PCR but the % is confusing. For example: 86.8% to 100% correspond to wet mount? 35.5% to 66% correspond to 18S rRNA or <i>pfoB</i> technique? Please clarify it. Lines 31 and 32. The author propose the PCR of <i>pfoB</i> gene as an alternative method for diagnosis of human trichomoniasis however the conclusion does not reflect the relevance of using <i>pfoB</i> PCR with respect to the other conventional methods. -Line 144. Method section needs to be completed such as the DNA extraction method. Please briefly describe it as in previous papers. -Reference list needs to be updated and completed. Please add references Moreno-Brito et al., 2005 and Meza-Cervantez et al., 2011 in addition to ref. 22. -How do you explain that no cross reactivity was mentioned against other trichomonad species? The reference they use to support their claim is referred to a patent that is not easy to access. Please show data that support this such as a multiple alignment of PFOs of Tv and of different species especially where the primers were made. Please also mention the % of similitude of the amplified region. -Data description is not clear, especially where the % of specificity, sensitivity and efficacy is mentioned. -Introduction and discussion sections show repetitive	Corrections done. Corrections done. Included latest references for this. Included these references as suggested. Included latest reference's which shows no



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	information. Please modify the text as need it. -Figure 1 needs more description in the text and figure legend. What does it represent? What the smear patters represent? Which are the Tv (+) and Tv (-) samples. Please complete the information and include the description of the results in the text. For example: -Figure 1. In panel A the lanes 1-4 and 6 show a negative amplification for 18S rRNA gene, but a positive amplification for <i>pfoB</i> gene. Please could you explain these results?. -Figure 1. In panel B the lanes 1, 3, 4 and 6 only show a smear. Are these lanes considered as a positive amplification for <i>pfoB</i> gene? What it is the possible explanation for these results? Line 145. Please include the TVAG_... number for each of the genes used in this study. Line 147. Please include the primers and briefly describe the PCR conditions used for the 18S rRNA amplification. -Are the primers used for <i>pfoB</i> gene amplification designed based on the variable region of the sequence? -The <i>T. vaginalis</i> genome revealed the presence of other <i>pfo</i>-like genes (<i>pfo-A</i>, <i>pfo-B</i>, <i>pfo-C</i>, <i>pfo-D</i> and <i>pfo-E</i>). How the specific amplification of <i>pfo-B</i> gene and no other <i>pfo</i>-genes was corroborated? -Please include DNA of human cells (HeLa, VEC cells, etc) as a PCR specificity control. -Lines 343-345. Based on your explanation, how do you expect the <i>T. vaginalis</i> detection in symptomatic women without metronidazole treatment? Do you expect 100% detection?	cross reactivity against other trichomonad species. (page 13, line no. 324) Done. Done. Done. Done. No they were considered negative. Done For this query we have included latest study by SC Sonkar et al 2016. Done. With our experiences, we can say that <i>T. vaginalis</i> can be detected 100% if right combination of two diagnostic methods are used for each samples.
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Minor REVISION comments	Lines 3 and 4. Please capitalize where it is needed. Example: “based” for “Based”, techniques for Techniques, diagnosis for Diagnosis, trichomoniasis for Trichomoniasis. Line 16 and line144. Please correct where it is needed. Is the range of patients’ ages “20-56” or “20-57”? Line 17. Please capitalize where it is needed: patients for Patients, department for Department, (OPD) for (OGPD), hospital for Hospital. Line 22. Please separate “<i>T.vaginalis</i> “ it should be “<i>T. vaginalis</i>”. Correct this word throughout the text. Line 42. Please change “quantum” by a more appropriate word. Line 44. Please change “reproductive age” for sexually active age. Lines 45-46. The phrase: “Clinical trichomoniasis in women, including symptomatic to an asymptomatic carrier state...” It is confusing. Please rewrite for clarity. Line 48. Please change “like” for such as. Line 136. “...normal saline...” What do you mean? For clarity, please explain or define this solution in the M & M section. Line 190. Please correct throughout the text the word “pruritis”. It should be “pruritus”. Line 214. Please correct the age range of “41-30 (n=82)” for “41-50”. Line 228. Please define “Ut” the first time you use it Line 240. Is (p=0.037) significant? Line 243. Are the percentages mentioned really significant for diagnosis of trichomonas infection? Line 328-330. Which of the two proteins PFO (PFO-A or PFO-B) show alternative function in adherence of <i>T. vaginalis</i> to host epithelial cells? Please include references Moreno-Brito et al., 2005 and Meza-Cervantez et al., 2011 to support your statement. Line 331. Please check the phrase “...the present study percentage positivity...”. It seems incomplete. Lines 342-343. Please explain how these percentages (6.25% and ~12%) were determined?	All corrections done. Done Done Done Done Done Done Done Done Done Done Done Done Done Done Done Done Done Done Done It was calculated as % of positivity for the DNA method used. Calculated as 21/344=6.1%; 7/56= 12.5% as shown in detail in Table 3. Thankyou for pointing it out. Appropriate correction is done in revised draft.
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	Ethical issues : Line 185. Please specify whether the consent was an oral or written consent obtained as previously mentioned in ref...	Done
<u>Optional/General</u> comments		