Author’s response to reviews

Title: Development of a polymerase chain reaction assay for the rapid detection of the oral pathogenic bacterium, Selenomonas noxia

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Author’s response to reviews: see over
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Dr. Marlise Klein
Associate Editor
BMC Oral Health
BioMed Central

Dear Dr. Klein,

Thank you for reviewing our manuscript entitled “Development of a polymerase chain reaction assay for the rapid detection of the oral pathogenic bacterium, Selenomonas noxia” (MS: 1354158762159719).

The authors have addressed the reviewer’s comments, and have enclosed a point-by-point response to their concerns. A revised manuscript is included with this submission (a version with track changes and one with changes accepted). We thank you for your consideration of our paper.

Sincerely,

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Development of a polymerase chain reaction assay for the rapid detection of the oral pathogenic bacterium, *Selenomonas noxia* (MS: 1354158762159719)

**Response to reviewers’ comments**

**Referee 1:**

Reviewer's report:

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

1. Table 6: It is not clear why the DNA input for each species was not normalized. If the input DNA were not equal, the results are difficult to interpret. If the PCR was not performed with equal input DNA for all strains, this should be performed.

   **Response:** The DNA input for each species was not normalized because the range of concentrations used was sufficient to obtain a positive result in a presence/absence assay based on the sensitivity of the reaction. The DNA concentration section of the Methods was revised to explain this rationale.

Minor Essential Revisions

1. Page 3, lns 8-19: Examples are given of the presence of *S. noxia* in disease, but how commonly is it found in healthy patients?

   **Response:** The literature and studies conducted to date do not give precise data on the overall prevalence of *S. noxia* in healthy individuals. Each of the studies that demonstrated a correlation of *S. noxia* to chronic periodontitis, generalized aggressive periodontitis, or sites converting from periodontal health to periodontal disease showed elevated levels of *S. noxia* in relation to the total bacterial population. In other words, *S. noxia* represents a greater percentage, and is found in higher proportions during these disease states. Our future studies using these validated PCR primers for *S. noxia* detection will aid in determining the overall prevalence of this organism in specific patient cohorts. The manuscript was revised to address prevalence in healthy individuals.

2. Page 3, lns 12-17: The wording makes it difficult to follow where one study ends and the next begins. Further, the statement “significant role in causing periodontitis” is an exaggeration of the results of the cited studies; the organisms mentioned were identified, but their association with a role in periodontitis was not investigated.

   **Response:** The reviewer is correct; the text has been revised, and additional references included.

3. Page 4, ln 2: The conclusion of the paragraph that begins with this sentence is awkward in this paragraph and would fit better at the end of the previous paragraph. The early part of this paragraph would fit better if combined with the paragraph that concludes the Introduction.

   **Response:** The introduction has been reorganized to present the relevance of the bacterium to oral health, the role of molecular methods in oral microbiology, and the association of *S. noxia* with obesity.

4. Page 4, ln 5: In what percentage of healthy individuals was *S. noxia* identified?
Response: The literature and studies conducted to date do not give precise data on the overall prevalence of S. noxia in healthy individuals; this is included in the revised manuscript as indicated above.

5. Page 4, Ins 12-13: This sentence is out of place within this paragraph.
Response: The authors agree with the reviewer; the sentence has been moved to the end of the 1st paragraph in the Background section.

6. Pages 5-6, Ins 27-7: As the “Template DNA concentration” section describes a standard technique, it should be shortened and combined with the “DNA extraction” section.
Response: These sections have been combined.

7. Page 6, Ins 15-20: As this approach was unsuccessful, it can be omitted from the Methods and left to the Discussion.
Response: As suggested, this approach was omitted from the Methods, and included in the Discussion.

8. Page 8, In 21: It would be nice if the data were performed at least in triplicate.
Response: Actually, all samples were analyzed in duplicate and experiments were repeated twice. The wording in the methods section has been revised.

9. Pages 9-10, Ins 24-5: “DNA concentration” is not a result. The paragraph should be removed. It is unclear what is meant by the “Selenomas noxia assay” and what sample templates were included. Were the input samples not normalized to include the same amount of DNA?
Response: This paragraph has been removed. The “Selenomas noxia assay” has been reworded as “Selenomonas noxia PCR assay”. The lower detection limit of the assay is based on S. noxia ATCC #51893, and this statement was moved to the PCR optimization section. As indicated in #1 above, the amount of template DNA in input samples was not normalized (and the text has been modified to make this rationale clear).

10. Page 10, Ins 21-22: Is it typical for IPC DNA to amplify so poorly? Cycle 29 is very late. Would the inhibition trends be different if a greater amount of input IPC DNA was used?
Response: It is typical for the IPC DNA to produce an average Ct value of 29. Cts < 29 are strong positive reactions indicative of abundant target nucleic acid in the sample (http://www.wvd.wisc.edu/wp-content/uploads/2013/01/WVDL_Info_PCR_Ct_Values1.pdf). We used the IPC as per the manufacturer’s instructions, and believe that if a larger concentration of input IPC DNA was used, the inhibition trends would be the same. This is because the IPC and target DNA both employ their own primers and probes. The manuscript was not revised.

11. Page 12, Ins 21-23: This control should be included in the methods/results.
Response: Words on use of the nanoCell to demonstrate the presence of DNA in negative non-target PCR samples were added to the “Internal amplification control” section in the Methods and to the “PCR inhibition” section of the Results.

12. Table 2: This table is confusing to follow. Type the full V8 region for each species, and make bold the bases that you wish to emphasize.
Response: The bases shown in Table 2 are the ones that the authors wish to emphasize as different. The table serves to illustrate the homology seen between these closely related species. While the table was not modified, a sentence was added to the Methods section to clarify the table.

13. Table 4: The differences resulted from the primer concentrations are quite minimal. This table should be eliminated and the summary of the results described in the text.
Response: Tables 4 and 5 have been removed, and the results are now described in the text.

14. Table 6: What is the mean concentration describing? Is this three independent replicates of DNA extracted from independent cultures? Or does it represent one extract from each culture whose concentration was read three times? If the latter, than the SE should be eliminated.
Response: The average shown in Table 6 represents one DNA extract from each culture whose concentration was read three times. This table has been omitted as indicated in #1 above.

15. Table 9: the SnP column can be eliminated and the results can be mentioned in the text.
Response: The change has been made as suggested by the reviewer.

Discretionary Revisions
1. As the goal of the authors was to develop a means by which to identify S. noxia in the oral microflora, the manuscript would benefit dramatically if the detection with the described primers had been performed on saliva and oral plaque samples. Detecting S. noxia in biological samples may prove to be more challenging than confirming from DNA isolated from pure culture.
Response: Thank you for your comment; the authors agree that testing of biological samples may be more challenging than testing pure cultures. Acquisition and testing of saliva and/or oral plaque samples is the next step for validation of the PCR assay developed but was not included in the objectives of this study.

Referee 2:
Reviewer's report:

The manuscript describes the development of a quantitative PCR assay to detect S. noxia. The topic is of interest. Overall, the manuscript is well written, but there as a couple of points for review to improve readability, as follow:
1. Please consider replacing the “Real Time PCR” by “quantitative PCR (qPCR)” throughout the text, as recommended by the MIQE guidelines (Bustin et al., 2009).
Response: Thank you for your comments. The change has been made as suggested by the reviewer.

2. Page 5, DNA extraction: please replace “Bacterial DNA” with “microbial DNA” because C. albicans is not a bacterium. Also, please double check the temperature at which the DNA samples were stored.
Response: The change to “microbial DNA” has been made. We verified that DNA extracts were stored at -70°C; therefore, the manuscript was not changed.
3. Page 7, … PCR optimization: what was the DNA concentration used as template to test primers and probe?

**Response:** *S. noxia* ATCC 51893 was used, and the total template per PCR reaction was 0.6 ng (600pg). The manuscript was revised to include this. The Primer Design section was also revised to clarify the strains used for testing.

4. Page 9, PCR optimization: Please comment on the primer concentration of 0.9 micromolar for both forward and reverse primers – this concentration appears to be somewhat high.

**Response:** This concentration is recommended by the instrument/software manufacturer, Applied Biosystems/Life Technologies, and is also indicated in the manuscript.

5. Page 9 and 10, DNA concentration: The data presented in table 6 is the yield of DNA recovered from the DNA isolation procedure. Were the DNA samples diluted to a specific concentration to serve as template for qPCR reactions?

**Response:** Dilutions of the template DNA were necessary to remove all PCR inhibitors present, but the samples were not all diluted to a specific concentration to serve as template for qPCR experiments (see response #1 to Reviewer #1 above). The manuscript was revised as indicated above.

6. Page 10, PCR inhibition: What is the possible source of PCR inhibitors? If the DNA samples were not diluted prior to qPCR reaction, could be that the high concentration of DNA templates were inhibiting the reactions.

**Response:** Previous research has shown the presence of PCR inhibitors in pure culture; therefore, the source of PCR inhibitors can be the microorganism itself. Our samples were diluted to “remove” the inhibitors; but high concentrations of DNA templates were not the source of inhibition. The Discussion addresses co-extraction of inhibitors with target DNA; the manuscript was not revised.