Reviewer's report

Title: Detection of hydrogen peroxide-producing Lactobacillus species in the vagina: a comparison of culture and quantitative PCR among HIV-1-seropositive women

Version: 1 Date: 11 April 2012

Reviewer: Camila Marconi

Reviewer's report:

Dear Editors,

Thank you for the opportunity of reviewing this manuscript. In fact, the narrow relation between vaginal flora and sexually transmitted diseases is a very interesting topic and studies in this field should be encouraged.

These authors aimed to evaluate the concordance of conventional culture and quantitative PCR for the detection of the potentially H2O2-producers: Lactobacillus crispatus and Lactobacillus jensenii in a cohort of HIV-1 seropositive women. A total of 376 samples from 57 women attending two services were included. Women were checked for HIV-1 blood load, vaginal infections and had culture and PCR results available in all visits. Their results shows that although there is a high detection rate of H2O2-lactobacilli producers by culture and L. crispatus and L. jensenii by quantitative PCR, the concordance between this technique is only moderate (0.45). Based on these findings they conclude that both methods present limitation, therefore they should be used combined for providing new information on vaginal microbiota.

The authors clearly described the importance and the aim of the study. The methods used are appropriate, although some additional information could be further included, as mentioned bellow. Nevertheless, the methods used are reliable and seemed to be very carefully performed. The results were shown in two tables that could be built in a more clearly form, as some data were difficultly found. Discussion is adequate but the conclusions in the abstract and the body of the text do not match. In fact, abstract could be improved as suggested bellow. In general, the writing is clear and correct. This article is not innovative, but should be considered for publication after some adjustments, as it was well performed and may provide important information for further studies on the phenotypes of bacterial species found in vaginal microbiota.

Major Compulsory Revisions

#1 In the conclusion paragraph of the abstract, the statements do not correspond to which I consider the main finding of the study - the moderate (Kappa=0.45 SD=0.05) concordance between culture and quantitative PCR and, thus, the recommendation to use both techniques combined according to the aim of further studies.

#2 Please provide inclusion and exclusion criteria used for women enrollment.
Were they sampled if they were menstruating? Under antibiotic therapy? Had recent sexual intercourse?

#3 Which tests were used for identifying presumptive Lactobacillus sp. strains? Were they submitted to Gram stain and catalase production? More importantly, how many presumptive Lactobacillus sp. colonies were selected from primary cultures for H2O2 assessment?

#4 Although authors state they assessed vaginal smears for flora classification, not much data on data are shown. More information should be included and compared to lactobacilli detection, which would enhance the findings of this study, mainly if as expected non-H2O2 producers were more frequent in BV women. Additionally, rates of BV, intermediate or normal flora should be provided per visit and not per women.

#5 In the exploratory analysis, how were the 16 out of 25 samples that met the inclusion criteria (#106CFUs) selected? This should be mention in the results section.

#6 Please adjust the layout of Table 1, as data are not clear enough, e.g. the number of culture-positive that tested negative by PCR can only be reached after calculation, which consists itself in a very important information that could be better displayed.

Minor Essential Revisions

#1 In the conclusion of the abstract, the statements how they were provided could be better incorporated in the discussion section.

#2 Please provide ATCC number in the standard strains used as positive controls, even that the methodology was previously described by these authors.

#3 If the broad range 16S rRNA primers used were not designed for this study, please provide a reference source.

#4 The term “BLAST algorithm” should replace “BLAST program”.

Discretionary Revisions

#1. As a quantitative PCR was performed, I would suggest that data on lactobacilli load of the cases PCR-positive but culture-negative could be and compared with PCR-positive but culture-positive ones. As a negative result on culture could easily explain a lower bacterial load.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests