Reviewer's report

Title: Vaginal flora in women from Greenland assessed by microscopy and quantitative PCR

Version: 1 Date: 16 August 2013

Reviewer: Michael Ferris

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Significance

Dactu et al. provide a detailed, generally well-written manuscript describing a study wherein 19 qPCR assays targeting 16S rDNA of known vaginal bacterial were used to assess the relative abundance of these species 177 women from Greenland who’s vaginal microbiota were sampled using self collected vaginal swabs and assessed for BV using Nugent Scores. The results showed that when qPCR measurements are stratified at certain threshold/cutoff levels, the results of the measurements of certain bacterial species are highly predictive of BV defined by Nugent Scores. Studies of bacterial species associated with BV, such as the one described in here, are important since the cause of BV is not completely understood, it is a common cause of vaginal irritation and it is associated with adverse pregnancy outcomes and increased risk of HIV infection. The study was approved by the Greenlandic ethics board and by the University of Toronto Research Board.

Discussion Section

Comment 1. Minor Essential.

Several other studies of BV using qPCR assays have been completed, including a recent PLoS paper by some of the same authors of the current manuscript, using several of the same qPCR assay on a different population of women. It seems reasonable that there should be some discussion of notable similarities/consistencies and notable differences, if any, between the results the current study and similar studies in the literature. A brief look at the ROC values in the tables in the PLoS paper and the current manuscript show remarkable similarities for some key species as indicators of BV in both studies.

Background Section

Comment 2. Minor Essential.

In the last paragraph of the Background Section, consider changing the word “introduced” to “used” or another term in the line …we introduced threshold quantification…because “introduced” can be taken to mean that this study was the first to take advantage of the relative abundance levels of bacterial species measured in qPCR assays to improve diagnosis of BV. The author’s recent PLoS publication and other studies cited in the reference section, Menard 2008,
Zozaya-Hinchliffe 2010 and others have shown the advantage of qPCR and quantitative measurements for more accurate diagnosis of BV.

Line 101 near the end of the Background section that reads “quantitative detection …which considers samples with a higher bacterial load than a previously established threshold or cut-off” … Please clarify to which “previously established” threshold or cutoff are the author’s referring? Does this mean threshold and cutoffs that were established by other researchers?

Methods Section

Comment 3. Major compulsory.

A description of negative controls is needed and methods used to control for contamination in qPCRs should be given.

Urine specimens were collected in GeneLock, a medium specifically designed to inhibit microbial growth and preserve nucleic acids for extended periods of time even at room temperature, while vaginal swabs, used for DNA isolation and qPCR were collected in UTM which contains ingredients designed to maintain viability and infectivity of chlamydia and mycoplasma. Vaginal swab specimens in the UTM were stored at 5°C for up to a week and were shipped at ambient temperature for an unspecified duration, and were then used for DNA extraction and quantitative PCR amplification of various vaginal bacterial species. The description of UTM does not seem to specifically indicate its ability to inhibit bacterial growth or DNA replication, or DNA preservation, were any controls performed, or are there any studies or product literature to cite, that validate that the relative concentration of vaginal bacteria species is unaffected by storage and shipping temperatures in UTM used in the study? Were any qPCR assays performed to assess whether bacterial concentrations in the UTM storage buffer change over time under the storage and shipping conditions? What was the number of negative controls included along with vaginal specimens for each batch of qPCR assays?


Beyond the inclusion of negative controls in each qPCR itself, please describe the precautions that were used to reduce the influence of bacterial contamination and bacterial DNA contamination in the qPCR assays? Were mock collections of sham swabs performed and included in parallel with vaginal swabs in DNA extraction and qPCR assays to assess the levels of 16S rRNA genes that might be detected by going through the motion of self-collecting vaginal swabs? Were negative controls included and taken through the DNA-extraction procedure along with vaginal specimens to check for contamination introduced during handling and cross contamination between DNA specimens? Perhaps such details were presented in previous publications by the authors and can be cited in the current manuscript? Or perhaps some tests can be performed to assess potential sources of contamination and this can be included in the manuscript.

Conclusion Section
Comment 5. Discretionary

Line 503. First line in the conclusions, consider changing the wording of the line “our study revealed a high prevalence of BV in women from Greenland” to our study confirmed a high prevalence of BV since it is stated in the introduction that other studies had also shown high (38%) BV prevalence in this population.

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

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.