

89. Bacterial Vaginosis multi-species biofilms: can standard quantification methods accurately quantify *in vitro* biofilms?

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Background. While it is well established that Bacterial Vaginosis (BV), the most common cause of vaginal discharge, involves the presence of a multi-species biofilm adhered to vaginal epithelial cells, in-depth study has been limited due to the complexity of the bacterial community comprising the biofilm. Assessing bacterial interactions between bacterial species that inhabit the BV biofilm can provide key information regarding synergism or antagonism between these species and provide insights into the pathogenesis of BV. Thus, proper biofilm quantification approaches are essential to further this body of research.

Objectives. To evaluate BV biofilm formation by several key individual BV-associated bacteria (*Gardnerella vaginalis*, *Fannyhessea vaginae*, and *Prevotella bivia*) and compare with a multi-species biofilm formed simultaneously by all three bacterial species.

Methods. Single- or multi-species biofilms were quantified by the crystal violet (CV) staining method, total cell counts by epifluorescence microscopy, and the plate counting technique (CFU); individual traits were assessed by bacterial species.

Results. Each individual species had a unique signature assessed by the distinct relationship between the total number of cells, CFU's, and total biofilm biomass.

Conclusions & Significance: The assessment of multi-species BV biofilm quantification results in significant bias, mainly since individual species quantification signatures can't be related to the multi-species consortia. To minimize this bias, a multiple-technical approach should be considered when quantifying multi-species BV biofilms, to circumvent the caveats of individual techniques alone, tailoring a more complete picture of the biofilm-forming capacity of key bacterial species and furthering the field of BV pathogenesis research.

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