Abstract #19: Characterization of meconium-associated microbiota by cpn60-based microbiome profiling underscores the need for robust negative controls in the study of low microbial biomass samples

Scott Jorge Dos Santos, MSc[1], Zahra Pakzad, MSc[2], Chelsea Elwood, MD[3,4], Arianne Albert, PhD[5], Janet E Hill, PhD[1], Deborah Money, MD[2,6] and The Maternal Microbiome Legacy Project Team, Other[2] (1)University of Saskatchewan, Saskatoon, SK, Canada, (2)Women's Health Research Institute, Vancouver, BC, Canada, (3)University of British Columbia, Vancouver, BC, Canada, (4)B.C. Women's Hospital & Health Centre, Vancouver, BC, Canada, (5)Women's Health Research Institute, Vancouver, BC, Canada, (6)The University of British Columbia, Vancouver, BC, Canada

Objectives: Many studies of placental, meconium or amniotic fluid microbiomes fail to account for the low-level contamination present in all sequencing experiments and do not implement adequate negative controls when interpreting results. We aimed to define and quantify the microbial content of meconium under properly controlled conditions.

Methods: As part of a larger study of the impact of the maternal vaginal microbiome and delivery mode on the infant gut microbiome, DNA was extracted from 142 meconium samples (the earliest stool specimen collected within 72 hours of birth) and subjected to *cpn*60-based microbiome profiling. Presence of amplifiable DNA was confirmed by human COX-1 PCR and total bacterial loads of meconium, sequencing controls and infant stool were compared by 16S rRNA qPCR. Culture was used to screen meconium for viable organisms and the clonality of the most frequently identified isolates was assessed by pulsed-field gel electrophoresis (PFGE).

Results: Low read numbers were obtained for most meconium samples, with PERMANOVA showing no significant differences in community composition between meconium and negative controls ($P = 0.267$). The 21 meconium samples containing higher read numbers were collected later after birth on average or originated from infants who exhibited meconium stained amniotic fluid during delivery. Total bacterial loads were significantly lower in meconium than in infant stool collected 3-months postpartum but did not differ from that of sequencing controls and correlated well with read numbers. Culture yielded 101 isolates from 59/142 (42%) meconium samples; the most frequently recovered organisms included *Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus epidermidis*. PFGE indicated a high level of strain diversity for *E. coli* and *S. epidermidis* isolates, and the presence of several identical strains of *E. faecalis*.

Conclusions: Meconium microbiome profiles could not be distinguished from exogenous contamination present in sequencing controls, highlighting the absolute requirement for robust controls in studies of low microbial biomass environments. Isolation of different bacterial strains from meconium underscored the contribution of each infant's unique microbial environment in the early stages of gut microbiome development.

Attachment: On next page

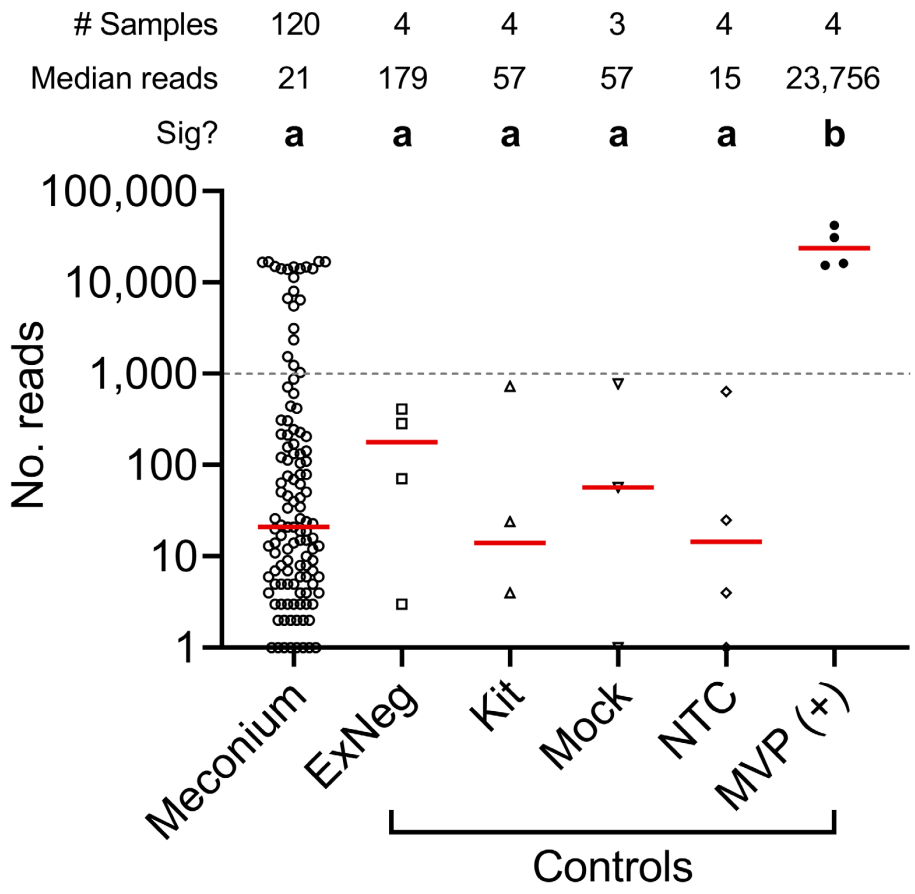

Figure: Number of *cpn*60 reads obtained from meconium samples, extraction negative controls, kit controls, mock samples, no template PCR controls and the mixed vaginal panel positive control (MVP). Only 21 meconium samples have read counts >1,000, indicated by a dashed line. Significant differences in read counts were assessed by a Kruskal-Wallis test with Dunn's multiple comparisons correction. Meconium read counts did not differ significantly from negative controls (a); however read counts from the MVP positive control were significantly higher than all other sample types (b; P <0.01).