RESPONSE TO REVIEWERS - 1 R01 AI140916-01

The review panel agreed that our "highly innovative [proposal is]…highly significant and…[would] potentially have high overall impact on STI management." Strengths included "focus on implementation to inform policy on STI testing strategies as well as cost assessment, the well-designed study, and strong investigative team." Weaknesses were the need for additional information and clarity; we thank the reviewers for their comments and have addressed them (in *blue underlined italics*) to strengthen our proposal, including:

Reviewer comment 1: "…unclear why study [includes] HIV+ and HIV- pregnant women…**Response 1:** updated Data Analysis section (Aim1). We agree the intervention should work regardless of HIV status. Recent work by our group[122] has shown that HIV infection independently predicts adverse birth outcomes. Given that and that 1/3 of South African pregnant women are HIV-infected, impact and effect size must be shown in both groups.

Reviewer comment 2: "…application lacks preliminary data for Aim 3. Persistent infection without treatment is different from treatment failure." **Response 2:** Added to the Preliminary Studies section: Effect of BV on Chlamydia Organism Load and Treatment Outcomes**.** As detailed there, our preliminary data suggest that altered vaginal microbiota impacts CT clearance and could impact CT treatment response.

Reviewer comment 3: "…wonders whether such a large sample size is necessary." **Response 3:** Our prior work found a large, significant effect size between prevalence of STIs at delivery in control and intervention arms. However, we were underpowered to detect differences in adverse pregnancy/birth outcomes. We powered this proposed study to detect differences in adverse birth outcomes, thus a larger sample size.

Reviewer comment 4: "… a DSMB is not proposed for this trial… **Response 4:** We agree. We updated the Data Safety and Monitoring Plan to establish a Data and Safety Monitoring Board (DSMB).

Reviewer comment 5: "overlapping roles with investigators" **Response 5:** We have added clarity about the distinct roles of investigators in the *Overall Structure of the Study Team* attachment of the human subjects section.

Reviewer comment 6: "My enthusiasm is dampened [by] …concern about…a standard of care arm…given that most STIs are asymptomatic" and "…equipoise" **Response 6:** Syndromic management is the standard of care in all low and middle-income countries. Demonstrating the impact/cost effectiveness of STI screening v. standard of care with respect to adverse birth outcomes is critical to produce high-level evidence to inform policy change.

Reviewer comment 7: "Dr. Klausner only has 10% effort" **Response 7:** Dr Klausner increased to 13-15% effort.

Reviewer comment 8: "Roll out of test and treat strategies is not in and of itself highly novel," and "significance and benefit of the proposed work is intuitive" **Response 8:** We seek to demonstrate the utility of STI test and treat strategies to reduce STI-related adverse pregnancy/birth outcomes, of which no significant literature exists. While we agree the benefit is intuitive, it is critical to measure effect size for costing models and implementation strategy, which has never been done before in a LMIC setting with point of care technology.

Reviewer comment 9: "Linkage of data to national databases can be challenging" **Response 9:** Prior work made extensive use of linkage to national datasets. We added a paragraph in the Preliminary Studies section, describing our prior successful use of national datasets.

Reviewer comment 10: "Enrollment table needs updating [re infants]" **Response 10:** Enrollment table updated.

Reviewer comment 11: "No discussion of space available within the two delivery hospitals to host research staff," "Research infrastructure at Tshwane clinics not well described," and "No documentation about medical records completeness to assess birth outcomes" **Response 11:** To address concerns, information has been added to the Study Setting section, describing space access, research infrastructure, birth outcome data collection/completeness, and related role of Co-I Pattinson (see Pattinson Letter of Support and biosketch).

Reviewer comment 12: "For Aim 3…metronidazole could alter the vaginal microbiota/modify the effect on CT treatment failure," and "it's unclear how many women treated with metronidazole and how that's factored into the analysis." **Response 12:** We use specimens from women with only CT in the Aim 3 microbiome analyses (no other antibiotic exposure). We will record metronidazole use and adjust for it in the other outcome analysis.

Reviewer comment 13: "Randomization procedure is vague" **Response 13:** Specific Aim 1, Recruitment and Eligibility**,** p6, now includes new detailed text on randomization procedures.

Reviewer comment 14: "The impact of HIV on vaginal microbiome and CT treatment response is unclear, and it's uncertain whether the study will be powered to investigate this." **Response 14:** Other covariates (HIV status, CD4 count, ART use) affecting the vaginal microbiome will be included in the statistical models to assess their effect on CT treatment success. While we powered the study to detect differences in intervention effects, we also have enough power to study the effects of covariates on outcomes.

Reviewer comment 15: "Inclusion/Exclusion of Children under 18: Including ages <18; not justified scientifically" **Response 15:** Revised criteria allow enrolment of those < 18.

Reviewer comment 16: "Cost-effectiveness model was thinly described; what data sources for disability and life expectancy outcomes among preterm children?" **Response 16:** We added Aim 2 modelling details and emphasize data sources.