SPECIFIC AIMS

Globally, an estimated 235,000 newborns are infected annually with HIV through maternal-to-child intrapartum/peripartum transmission.[1] While "Option B and B+" HIV treatment programs for pregnant women are increasingly scaled up, even in well-performing programs mother-to-child transmission (MTCT) of HIV still occurs.[1] In order to reach the UNAIDS goals of zero new infections and the elimination of MTCT of HIV infection, co-factors that increase MTCT of HIV infection must be addressed.[2]

Recent research by our group from a sub-study of NICHD HPTN 040 demonstrated that the sexually transmitted infections *Neisseria gonorrhoeae* (NG) *and Chlamydia trachomatis* (CT) conferred a 2.6 times increased risk of mother-to-child HIV transmission.[3] In that sub-study of 1373 HIV-positive pregnant women tested for NG and CT, 24.2% of the HIV MTCT was attributable to NG and CT co-infection.[4] Our work confirmed a prior 2001 study in Tanzania demonstrating that among HIV-infected pregnant women co-infection with NG was associated with a 5.5 times increased risk of intrauterine HIV transmission.[5] NG and CT have long been known to increase the sexual transmission of HIV through their effects on genital inflammation, increased genital HIV viral load and increases in target cell activation, but no research has explored the role and potential impact of screening and treatment of NG and CT infections in maternal-to-child HIV transmission in the modern era of treating HIV-infected pregnant women with combination antiretroviral therapy.

The WHO estimates there are 105.7 million and 106.1 million new cases annually of CT and NG infection, respectively, making these infections the most common bacterial sexually transmitted infections globally.[6] Large studies using current molecular-based diagnostic technology documenting the problem of CT and NG in pregnant women are lacking in Africa, and those existing suggest similar if not higher CT and NG prevalence than those reported by the WHO.[7-11] HIV-infected women have higher rates of CT and NG infection than HIV-uninfected pregnant women.[12] The singular focus on MTCT HIV prevention has resulted in the neglect of CT and NG treatment in pregnant women and missed opportunities to reduce devastating STI-related adverse pregnancy outcomes including the impact of STIs on MTCT of HIV infection.[12-15]

South Africa has an estimated preterm delivery rate of 8 per 100 live-births, resulting in more than 80,000 preterm births annually. Preterm birth is associated with about 60% infant mortality.[16] In addition, with one of the largest number of HIV-infected pregnant women delivering annually in the world (>300,000),[17] both adverse birth outcomes and MTCT HIV are significant public health problems; however, few studies have systematically measured the role of STIs and adverse birth outcomes in HIV-infected South African women.

In response to the need for further research to eliminate MTCT of HIV infection and reduce infant morbidity and mortality, we propose a study to investigate the feasibility and acceptability of CT and NG screening in pregnant women and the potential impact of such a screening program on the MTCT of HIV infection. Two antenatal clinics in Tshwane District, Gauteng Province, South Africa, will provide a unique setting to comprehensively study those issues in further detail.

Our proposed project has the following two Specific Aims:

Specific Aim 1: To determine the acceptability and feasibility of screening and treating HIV-infected pregnant women for NG and CT at first antenatal care visit.

1(a): To estimate the prevalence of CT and NG in HIV-infected pregnant women attending their first antenatal visit in Tshwane District, South Africa

1(b): To examine correlates of prevalent CT and/or NG infection and CT/NG treatment outcomes among pregnant women in the study

1(c): To determine the proportion of eligible women consenting to testing (acceptability) and receiving treatment (feasibility)

Specific Aim 2: To describe longitudinal birth and infant outcomes for HIV-infected pregnant women screened for CT and NG in their first antenatal care visit.

2(a): To estimate the frequency of adverse birth outcomes such as premature rupture of membranes, preterm labor or delivery, and small for gestational age, and their association with CT and NG screening and treatment

2(b): To estimate the frequency of HIV MTCT and its association with CT and NG screening and treatment

As South Africa aims for ZERO new HIV infections from MTCT, identifying and eliminating putative risk factors to MTCT is of utmost importance. The collaboration between the Foundation for Professional Development (FPD) South Africa and the UCLA Program in Global Health will improve local capacity to conduct high-level research and epidemiological studies, ultimately strengthening South Africa's ability to contribute to the global body of biomedical and public health knowledge.

RESEARCH STRATEGY

A.SIGNIFICANCE

HIV and STIs among pregnant women in South Africa are a critical problem. In 2011, the most current year for which data are available, the South African government estimated that 29.5% of women seeking antenatal care (ANC) were HIV-positive,[18] a prevalence that has remained relatively stable since 2007. Such high levels of HIV prevalence are further compounded by the high rates of STIs in women of reproductive age, and more specifically, pregnant women. A recent study conducted in Tshwane District, Gauteng Province, found that 26% of women presenting at a termination of pregnancy clinic had single, double or triple infections of CT, NG, or *Trichomonas vaginalis* (TV).[19] Infants in South Africa routinely receive chloramphenicol antibiotic eye ointment at birth to prevent neonatal bacterial conjunctivitis, most often caused by untreated maternal chlamydial or gonococcal infection.[20] Yet the risks to infants are greater than this; our recent analysis in a sub-study of NICHD HPTN 040[3] highlights the increased risk of HIV MTCT in the presence of dual CT/NG infection:

Table 1. HIV MTCT and CT/NG Co-infection, South Africa, Brazil, Argentina & US, HPTN 040 Cohort

Characteristic	CT/NG co-infection	CT/NG uninfected	RR of MTCT	PAF[4]	P-value
HIV+ pregnant women	25	800			
HIV MTCT	5	62			
Percent HIV transmission	20.0%	7.8%	2.6 (1.1 – 5.8)	24.2%	<.0001

Given that most gonococcal and chlamydial infections in women are asymptomatic, and that South African government health programs do not routinely screen asymptomatic pregnant women for such STIs, the true burden of disease from STIs in this population is likely even higher than statistics suggest.

MTCT may be associated with genital tract HIV shedding, CT, and NG. It has been hypothesized that co-existing bacterial STIs (CT and NG) in pregnancy may impact HIV maternal-to-child transmission.[21] However, to date few studies have investigated the interactions of those STIs during pregnancy and their effects on vertical HIV transmission. Prior research in non-pregnant women has suggested that co-infection with STIs in HIV-infected women may augment the risk of HIV transmission by increasing viral shedding,[22-25] and subsequent treatment of these STIs can reduce the risk of HIV transmission.[26,27] Limited existing research has suggested that the presence of STIs in HIV-infected pregnant women may increase the risk of HIV MTCT. One study of HIV-infected women in Tanzania reported that co-infection with NG was associated with a 5.5-fold increased risk of intrauterine HIV transmission.[5]

There continues to be room for improvement with PMTCT and Option B in South Africa. In 2004-2005, it was estimated that AIDS contributed to about 40% of all child deaths under age five in South Africa.[28] Prior to this in 2002, a national PMTCT program had been implemented, but was poorly resourced with resultant poor population coverage. In 2008 the South African government launched the national PMTCT Accelerated Plan (Option A). While the number of HIV-exposed infants remained stable (230,000-240,000) between 2008 and 2010, the number of infants with a positive HIV PCR result dropped from 9.6% to 3.5%, respectively, with a MTCT rate ranging across the provinces from 1.4% to 5.9%.[29,30] Though tempered by a low (35.1%) uptake of early infant diagnosis testing, significant progress has been made in nationally enhancing coverage of PMTCT services. However, significant variability remains in PMTCT service coverage and quality nationally, with structural barriers and individual health decisions continuing to impact access and uptake. In 2013, the South African National Dept. of Health updated their PMTCT guidelines, hewing closely to the WHO's Option B recommendations. Though policy and operational enhancements have strengthened the national PMTCT program, MTCT of HIV still occurs and in some provinces is higher than 5%.[31]

B. INNOVATION

The current proposed study will help fill critical gaps in the understanding of how bacterial STIs may impact MTCT of HIV infection in the era of combination ART in pregnant women. Given the high prevalence of HIV infected pregnant women in South Africa (over 300,000 HIV-infected women deliver annually[17])and the high prevalence of STIs in women of reproductive age**,** South Africa provides an ideal setting to understand these multifaceted, overlooked interactions. At present, little is known about the ways in which bacterial STIs in pregnancy may impact MTCT of HIV. An enhanced, comprehensive understanding of risk factors for HIV MTCT is essential, particularly in South Africa with its high prevalence of co-infection.

Currently, prenatal screening for bacterial STIs is not routinely conducted in low and middle-income countries around the world. While South African policy stipulates that pregnant women are to be screened for HIV and syphilis during their first ANC visit, routine antenatal screening is not conducted for CT or NG. Studies such as this one may help enhance our understanding of the prevalence, impact and attributable risk of CT and/or NG

infections and MTCT of HIV. Furthermore, given the known adverse consequences of CT and NG on maternal-child health outcomes, this study may be able to directly inform public health programs and policy to improve the health and wellness of women and children. Specifically, potential interventions include the addition of CT and NG screening as part of the first ANC visit in South Africa. Finally, in high risk populations such as pregnant women with HIV, screening for CT and NG may have additional benefits by decreasing the risk of preterm birth, low birth weight, neonatal conjunctivitis, pneumonia and infant death.

This study is novel and innovative in 3 primary ways:

1) This pilot study is designed to determine the feasibility and acceptability of routinizing CT/NG screening and treatment of HIV-infected pregnant women attending ANC visits using the recently FDA-cleared commercially available point-of-care CT/NG molecular assay [Xpert CT/NG, Cepheid, Sunnyvale, CA]. Molecular CT/NG screening is not currently available in most low and middle-income countries globally; however, the Cepheid Xpert CT/NG assay is easy to use and allows for decentralized, non-laboratory-based clinic test. It is ideally positioned for uptake in low and middle income settings. The Cepheid Xpert testing platform is already widely deployed in southern Africa and used for rapid diagnosis in tuberculosis. The addition and use of another test cartridge (CT/NG) is quite feasible.
2) The study findings will enhance knowledge of the prevalence of maternal and congenital infections as well as related birth outcomes in high risk populations in South Africa. While preliminary research including work by this study team has demonstrated an association between bacterial STI infection and poor birth outcomes, these interactions are not yet widely understood and thus evidence to support efforts to prevent and treat CT and NG in pregnant women in low and middle-income countries is urgently needed.
3) The study findings will enhance knowledge about how CT and NG may influence MTCT of HIV, especially for pregnant women in high prevalence populations. As PMTCT programs continue to increase and improve throughout the world, too often these efforts are narrowly focused. A more comprehensive understanding of the role of co-infection with bacterial STIs and the impact this has on MTCT of HIV will serve to greatly improve the effectiveness of these HIV PMTCT programs.

For the reasons outlined above, this study has the potential to significantly inform programs aimed at the prevention of HIV MTCT in South Africa in the era of Option B policy, and to directly impact clinical and public health practices in low and middle-income countries relating to maternal-child health, especially relating to bacterial STIs. If shown to be feasible, acceptable, and potentially efficacious, the pilot intervention from this study will serve as a basis for larger controlled trials in the future.

C. APPROACH

The Foundation for Professional Development (FPD) has a standing Memoranda of Understanding with the Gauteng Provincial Department of Health to support clinic-based health systems strengthening in Tshwane District (see Appendix 1). As such, this study will leverage our already strong relationship with both provincial and district health departments to develop additional clinical sites for PMTCT research and program development.

C.1. Overview and Timeline. This study encompasses 3 phases, as detailed in Table 2 (next page):

- ***Phase I:*** Development and piloting of recruitment, enrollment, specimen and data collection tools, study staff training and finalization of screening, laboratory, and treatment protocols
- ***Phase II:*** Recruiting and enrolling 600 intervention participants and 600 participants in a non-intervention comparison group; intervention will include laboratory testing, appropriate care/treatment of STI infected pregnant women, and patient follow-up including test of cure and assessment of birth/ infant outcomes
- ***Phase III:*** Data analysis, dissemination of findings, and preparation for future research

Table 2: Study Timeline

Component and Task Name	Start	Finish	Duration	Year 1				Year 2			
				Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
I. Preparation and Tool Piloting	Apr 2015	Sept 2015	6 mo.								
II. Participant Enrollment and Follow-up	Oct 2015	Sept 2016	12 mo.								
III. Data Analysis and Dissemination	Oct 2016	Mar 2017	6 mo.								

C.2. The Study Setting. This study will take place in two antenatal clinics in Tshwane District (Pretoria), Gauteng Province, South Africa: 1) KT Motubatse Clinic, which in 2013 had a monthly head count of 105 first time ANC visits, and a maternal HIV positivity rate of 31.4%; and 2) Soshanguve Community Health Center, which had a 2013 monthly head count of 149 first time ANC visits, and a maternal HIV positivity rate of 29.9% (District Health Information System, 2013). A letter of support for the Tshwane District Department of Health, agreeing to commit clinic resources to this project, is included with this proposal.

C.3. The Research Team. **Jeffrey Klausner, MD, MPH** (UCLA Co-PI): Dr. Klausner is an infectious disease epidemiologist and Professor of Medicine and Public Health in the UCLA Division of Infectious Diseases, School of Medicine and the Department of Epidemiology, School of Public Health. This study builds on more than 20 years of prior STI screening and treatment studies in San Francisco, South Africa and Peru. As Director of STD Prevention and Control Services in San Francisco, 1998-2009, Dr. Klausner began studies investigating the performance of molecular STI diagnostics, the role of self-specimen collection for STIs and the introduction and evaluation of population-based screening programs in schools, jails and adolescent clinics.[32-39] From 2009-2011 Dr. Klausner was Branch Chief for HIV and TB at the US Centers for Disease Control and Prevention in Pretoria, South Africa, helping lead the South African PEPFAR program for PMTCT, HIV care and treatment. In that role Dr. Klausner worked directly with national, provincial and district health authorities and local South African non-governmental organizations including FPD to support the scale-up of PMTCT services nationally. He played a key role in describing the population-based provincial rates of MTCT as part of the South African national PMTCT effectiveness evaluation.[29,40-42]Dr. Klausner is a member of the WHO STI Guidelines Committee and frequent advisor to ministries of health on HIV and STI prevention. He will have 0.20 FTE on this project, and will provide oversight of the research design, implementation, and analysis.

Andrew Medina-Marino, PhD (FPD Co-PI) is Head of FPD's Research Unit and Senior Technical Advisor for Laboratory and Disease Surveillance Systems Strengthening activities. Previously, Dr. Medina-Marino was Laboratory Branch Chief for CDC-South Africa. In this capacity, he supported and advised the South African National Institute for Communicable Diseases (NICD) National Health Laboratory Service (NHLS), South Africa's national pathology service provider, on the expansion of laboratory based surveillance programs. He also worked directly with NHLS and the National Department of Health to develop national point-of-care policy and guidelines. As a Molecular Biologist, Dr. Medina-Marino helped identify a key cell receptor that facilitates NG adherence and invasion.[43] Dr. Medina-Marino has worked extensively with the Tshwane District Dept. of Health and the staff at the two clinics that will serve as study sites. For this project he will provide direct oversight for the South African-based study team, including study nurses who will have direct contact with patient-participants and data managers. In his role as Co-PI he will devote 0.20 FTE and will oversee and ensure quality of all in-country study implementation efforts.

Joy Ikechi Ebonwu, MPH (Co-Investigator): Ms. Ebonwu is a STI Epidemiologist with FPD. Prior to working for FPD Ms. Ebonwu was an epidemiologist with the Centre for HIV and STIs at the National Institute for Communicable Diseases within the National Health Laboratory Service in South Africa. Ms. Ebonwu is currently assisting in the development and implementation of the first national sentinel STI etiological surveillance project. She has also worked as a laboratory supervisor at National Health Laboratory Service Mycobacteriology referral laboratory in Braamfontein, Johannesburg and has spent considerable time performing diagnostic procedures at the STI Research Laboratory at George Mukhari Hospital in Pretoria. Ms. Ebonwu is a graduate of the South African Field Epidemiology and Laboratory Training Program and has a Master of Public Health from the University of Pretoria and a Master of Medical Microbiology from the University of Witwatersrand in Johannesburg. For this project, Ms. Ebonwu will provide coordination and oversight of all specimen collection, and laboratory processing of all study specimens at both study sites.

Xiaoyan Wang, PhD (Co-Investigator): Dr. Wang is an Assistant Professor within the Statistics Core of the UCLA Department of Medicine. She has extensive experience with biostatistics in the design and analysis of large-scale cohort, cross-sectional and intervention studies. She will be responsible for all statistical analyses for this project.

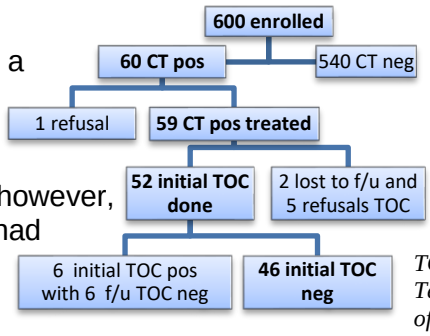
James McIntyre, MBChB (Consultant): Dr. McIntyre is an OB/GYN physician-scientist and internationally-recognized expert on HIV MTCT. He is the executive director of the Anova Health Institute, and the international vice-chair of the NIH-funded International Material Paediatric and Adolescent AIDS Clinical Trials Network (IMPAACT). He regularly serves as a consultant on these issues to the WHO, UNAIDS, and UNICEF. He will provide approximately 2 hours of consultation each month for both years of this project.

C.4. Preliminary Studies. NICHD HPTN 040 sub-study. The NICHD HPTN 040 parent trial was a randomized multi-center clinical trial in Brazil, South Africa, Argentina, and the U.S. that enrolled 1684 HIV-exposed infants from HIV-infected mothers without prior antiretroviral use.[44] In our UCLA sub-study (Klausner, PI), we used remnant urine specimens from the pregnant female participants, tested those for CT/NG infection using the testing platform we will use in this proposed study [Xpert CT/NG, Cepheid, Sunnyvale, CA], and described the prevalence of CT and NG infections. Among the 409 pregnant South African participants the prevalence of CT was 21.3% and NG, 7.6%. Women with dual CT and NG infection were twice as likely to vertically transmit HIV than those without either infection.[3]

Pilot Study: Lima, Peru, Nov 2012 – May 2013. Most recently, the UCLA team (Klausner, PI) completed a large acceptability and feasibility study of CT screening among pregnant women (N=600) in ANC at 2 large urban hospitals in Lima, Peru.[45,46] Over a 2 month period, 640 patients were approached and 600 enrolled (approximately 300 patients/month with a 94% enrollment rate and 98% treatment rate; See Figure 1). The average age of women participants was 27.3 ± 6.8 years (range 16-47) with an average of 2.3 ± 2.6 lifetime partners (range 1-50), and an average gestational age of 26.3 ± 10.6 weeks (range 4-41). CT prevalence in our study population was high at 10% (95% CI 7.7 – 12.7%). Prevalence decreased with age, with women 16-23 years having the highest prevalence (15.6%), and the lowest prevalence in women ≥ 31 years (5.2%).

Overall, 59 (98%) of the 60 pregnant women found to be CT positive were treated with azithromycin (1 refused), and 52 (88%) returned for test of cure; all (100%) of these women were found to be treated successfully. CT screening and treatment in pregnancy was both feasible and highly acceptable in this patient population with high CT prevalence.

Fig. 1: CT in pregnancy screening/ treatment participant flow diagram, Lima, 2013.



TOC = Test of Cure

Maternal and infant outcome data were also collected on a retrospective convenience sample of 249 patients of the pilot study (see Appendix 2 for details on outcomes). The median gestational age was 39 weeks; with preterm delivery rates of around 6%. Of note, data were not collected on preterm labor rates; however, premature rupture of membranes was 3.6%. 1.6% of patients had low birth weight, with one stillbirth (0.4%).

C.5. Methodology and Study Aims.

Specific Aim 1: To determine the acceptability and feasibility of screening and treating HIV-infected pregnant women for NG and CT at first antenatal care visit.

Methods and Procedures

In order to accomplish Specific Aim 1 we will conduct a cross-sectional study among HIV-infected pregnant women who are receiving ANC at one of our two collaborating clinic sites. Through this we plan to achieve two subaims: 1(a): To estimate the prevalence of CT and NG in HIV-infected pregnant women attending their first antenatal visit in Tshwane District, South Africa, and 1(b): To examine correlates of prevalent CT and/or NG infection and treatment outcomes among pregnant women in the study.

Recruitment and Eligibility: We will recruit 600 study participants from pregnant women presenting for ANC services at the two study clinics in Tshwane District, Pretoria, South Africa. Together those 2 clinics see about 250 new women each month for ANC. Eligible criteria include: 1) Age ≥ 18 years, 2) Currently pregnant, 3) Documented HIV infection, 4) Attending the first ANC visit for this pregnancy, 5) Willingness to self-administer a vulvo-vaginal swab, 6) Residence in Tshwane district, and 7) Intent to stay in Tshwane district through delivery.

Clinic staff and study recruiters will be trained in the study methods and human subjects' research. They will also receive training on South Africa's syndromic management algorithms for STIs. Patients will be preliminarily screened for eligibility via chart review at the time of the appointment; all those suspected to be eligible will be formally screened during the visit by study nurses. All eligible patients will be provided specific information about CT and NG infection, the consequences and treatment of those infections, study risks and benefits, and invited to participate. Those providing informed consent will be enrolled, instructed on how to self-collect a vaginal swab specimen and asked to share several forms of detailed contact information (e.g., personal, family, friend, residence, and work) to assure follow-up. Women who are currently pregnant with documented HIV infection but otherwise ineligible will be logged with reason for ineligibility; data will be used for descriptive analysis of the differences between our study population and the general ANC patient population.

A data collection instrument (see Appendix 3) will be used to collect demographic, sociobehavioral characteristics, knowledge related to CT/NG/HIV effect on pregnancy, pregnancy history, and other relevant clinical information. Those data will be abstracted from patient medical records and interviews by study nurses.

Acceptability and feasibility. Acceptability of NG and CT screening at the first ANC visit will be defined as at least 80% of eligible women offered CT/NG testing consenting to testing. Feasibility will be defined as at least 90% of all pregnant women who test positive for CT and/or NG through the pilot screening program provided standard treatment per South African STI Treatment Guidelines[48] and returning for test of cure.

Specimen Collection, Transport, Processing and Storage: Eligible participants will be asked to provide a self-collected vulvo-vaginal swab specimen during their visit. Specimens will be handed to a trained nurse who will label them with a unique study barcode and place them in a secure storage area for up to 24 hours at 2°C to

30°C until tested. Remnant specimens will be batch frozen at -80°C, and discarded within 6 months after data collection is complete, according to Good Laboratory Practice (GLP).

Laboratory Testing: Specimens will be tested for NG and CT using Xpert® CT/NG [Cepheid, Sunnyvale, CA] at each of the clinical sites by trained technicians. The vaginal swab used for testing is contained in the Cepheid GeneXpert CT/NG Vaginal/Endocervical Specimen Collection kit. Once collected, the study technician will follow the test kit instructions for swab preparation and testing. Xpert provides 90-minute detection and differentiation of CT and NG; it has high sensitivity and specificity[47] and functions well in resource-constrained environments and clinical settings such as those proposed here. All specimens will be run on-site with standard controls, per manufacturer's instructions. Each test includes a sample processing control (SPC) to ensure correct processing of the sample, a sample adequacy control (SAC) which ensures adequate cell lysis for nucleic acid extraction and a probe check control (PCC). The PCC monitors reagent rehydration, reaction-tube filling, probe integrity, and dye stability. Ms. Ebonwu (Co-Investigator) will oversee laboratory training and testing to ensure high quality and reliability of results.

Reporting and Treatment: The Xpert system consists of an instrument, personal computer, and preloaded software for running tests on collected samples and displaying results as either positive or negative. All study participants testing positive for either CT or NG will be immediately notified by the study nurse. Appropriate clinical staff will provide standard care and treatment per the South African Department of Health's STI treatment protocols.[48,49] Study participants being treated for CT and/or NG will be asked to provide a second vaginal swab specimen 3 weeks after treatment to document treatment outcome. Final treatment outcomes will be assessed by collection and testing of post-treatment vaginal swab specimen. Study nurses will record testing results in the patients' clinical file as well as into report forms designed for the study.

Analysis: Proportions of NG and CT infection in HIV-infected pregnant women will be based on positive PCR test results [# positive / (# negative + # positive)].Treatment outcomes will be calculated as the proportion of treatment success vs. treatment failure. "No treatment" will be categorized as failure.

Potential Challenges and Quality Assurance: Loss-to-follow up of participants testing positive for CT or NG and test turn-around-time (TAT) will likely be the dominant challenges in this study. As part of the study, FPD will leverage existing clinical roving teams and contact tracers to work directly with the clinic-based study nurses to find lost patients. Clinic and laboratory study personnel at each study site will meet twice monthly to review study enrollment, specimen collection, processing, TAT, data management, and treatment outcomes. Meetings will discuss descriptive study results to date, problems encountered and remedial actions to be taken.

Specific Aim 2: To describe longitudinal birth and infant outcomes for HIV-infected pregnant women screened for CT and NG in their first antenatal care visit compared with those unscreened for CT/NG.
Methods and Procedures.

At the time that participants are being enrolled into the study as part of Aim 1 above, 600 additional participants will be enrolled as part of a comparison group in order to achieve Aim 2. Birth outcome data will then be collected from the 600 participants participating in CT/NG screening as part of Aim 1 as well as the 600 women enrolled in the comparison group. Data on birth outcomes will be used to accomplish two subaims: 2(a): To estimate the frequency of poor birth outcomes such as premature rupture of membranes, preterm labor or delivery, and small for gestational age, and their association with CT and NG screening and treatment, and 2(b): To estimate the frequency of HIV MTCT and its association with CT and NG screening and treatment.

Recruitment and Eligibility: The 600 women in the comparison group will be recruited and enrolled similarly to those participants enrolled in Aim 1. Eligibility criteria for the comparison group are identical to those in the screening group (see Aim 1) except they will have previously attended their first ANC visit and thus be ineligible for screening, but be at least 4 weeks from anticipated delivery. The participating clinics do not have access to ultrasound machines; therefore, per South African guidelines gestational age will be measured using symphysis-fundal height (SFH). The SFH measurement will be plotted onto the 50th centile line on the SFH graph, allowing the corresponding GA to be read from the graph. As with the screening group, patients will be preliminarily screened for eligibility in the comparison group via chart review at the time of the visit; those suspected to be eligible will be formally screened during the visit by study nurses. All eligible patients will be invited to participate, and those providing informed consent will be enrolled.

Data Collection: Study staff will collect data on adverse pregnancy events in study participants of both the screening and comparison group through face-to-face interviews within 2 weeks of delivery and by review of medical records (see draft data collection instrument, Appendix 4). Staff will collect information on fetal loss, preterm labor, preterm birth, birth weight and small for gestational age status, as well as infant health data including mortality and serious adverse events including respiratory distress and conjunctivitis. Information on other potential confounding variables such as a maternal history of chronic illness (i.e., hypertension, diabetes),

other infections during pregnancy (i.e., urinary tract infections, syphilis), antibiotic usage during pregnancy, and pregnancy complications (i.e., premature rupture of membranes, maternal fever, chorioamnionitis, pre-eclampsia) will also be collected. At 7-8 weeks post-delivery, both HIV PCR test results from routine early infant diagnosis (EID) of HIV-exposed infants at six weeks of age and evidence of chlamydia pneumonia will be accessed via clinic records by the clinic study nurse or other FPD clinical staff with appropriate permission to access patient medical records (see draft data collection instrument, Appendix 5). Data collection will be reviewed weekly by a study supervisor who will ensure the completeness and validity of the data by comparing participants' reported outcomes with clinic records; discrepancies will be resolved via interview with the birth attendant (midwife or physician).

Retention and Follow-up: To ensure post-delivery follow up, multiple forms of contact information will be collected for all participants at enrollment. To develop and maintain a strong relationship with study participants, study nurses will check in with each participant during monthly, regular pickup of antiretroviral therapy. To further support active follow up, participants will also be encouraged to enroll in the UNICEF-funded MomConnect program, which sends reminder messages to pregnant women's mobile phones via text messages to remind them about their upcoming ANC visits, and automatically generates alerts for clinic staff when appointments are missed. Participants who do not return for scheduled ANC or ART visits after enrollment will be actively contacted by both clinic and study staff and encouraged to return for care. All participant charts will be flagged so that study staff will be notified at the time of delivery. Seven days post-delivery study staff will contact participants to schedule an outcomes interview. Up to seven attempts will be made through various contact methods (i.e. text, mobile phone call, home visit) to follow up with participants. Our prior studies have achieved retention rates greater than 98%[46] and similar rates are expected in this study.

Data Analysis: MTCT data analysis will focus on a pooled, estimate of the effect of CT and NG screening and treatment on MTCT of HIV. The individual effects of screening/treatment for each of these infections will be evaluated with respect to the infants' HIV status. Particular focus will be placed on whether HIV-infected mothers who are unscreened for both CT and GC are at higher risk of having HIV-infected infants (non-intervention comparison group). We will analyze birth outcomes with a particular focus on predictors of preterm birth (babies born alive before 37 weeks gestation) with sub-categorization as extremely preterm (<28 weeks), very preterm (28 to 32 weeks), and moderate-to-late preterm (32 to 37 weeks).

Potential Challenges: Loss-to-follow up for EID testing of HIV-exposed infants will likely be the main challenge with this Aim. FPD will leverage existing clinical roving teams and contact tracers to work with the clinic-based study nurse to ensure EID testing. It is also possible that deliveries could occur in township settings without a medical birth attendant; after the expected time of delivery, study staff will work to contact participants and will travel to their township to collect birth outcomes if needed. A recent evaluation study found that when coordinated with 6-week immunization visits, infant EID follow-up was >90%[31]; this strategy has since been adopted at all FPD-supported clinics and we expect to meet or exceed this follow-up rate.

C.6. Sample Size Estimations and Statistical Analyses

Sample Size. The sample size for Aim 1 will be 600 women and the sample size for Aim 2 will include 1200 women total (the 600 participants from Aim 1 and an additional 600 participants in the comparison arm). This sample size was chosen based on the regular head count of patients for ANC visits at the participating clinics, as well as the need for sufficient study power. With 600 eligible subjects in Aim 1, we will be able to estimate a test consenting rate of 80% (acceptability) to within a 95% confidence interval of +/- 3.3%. Based on the pilot study, we expect 120 participants to test positive for CT and/or NG (20% out of 600). A two-sided 95% confidence interval for 90% of these 120 women being treated (feasibility) will have a width of 11.6%. Similarly, a two-sided 95% confidence interval for 80% of the test results report within a week (feasibility) will have a width of 6.6%. The proposed sample size will provide enough precision for valid estimation of these quantities.

Statistical analysis. Descriptive statistics including mean, standard deviation, median, inter-quartile range and frequency distribution will be generated for outcome variables as well as provider and patient characteristics. Graphics such as bar charts, box-plots, and histograms will be used to present the data and check for skewness and normality. Transformations of the outcome variables will be explored and performed if needed. For Aim 1, proportions related to acceptability and feasibility and the corresponding 95% confidence intervals will be calculated. For Aim 2, propensity score method (matching, stratification, or weighting) will be used to adjust for possible confounders when evaluating birth outcomes between women with CT and NG screening and treatment and the control group. Statistical analysis will include determination of prevalence, adjusted odds ratios, confidence intervals, and multivariate logistic regression. Longitudinal birth outcomes will be described by frequency estimates of single events and multiple events. For all statistical investigations, tests for significance are two-tailed. All analyses will be conducted with Stata 9.0 (Stata Corporation, College Station, TX, 2006).

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