Title: The diagnosis and treatment of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in pregnant women to prevent adverse neonatal consequences.
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Protocol Contents

1. Issue:

Chlamydia trachomatis (CT), *Neisseria gonorrhoeae* (NG), and syphilis (caused by *Treponema pallidum*) are bacterial sexually transmitted infections (STIs) and are neglected causes of adverse neonatal outcomes. According to WHO modelling estimates, CT, NG, and syphilis are among the most common STIs globally. These infections are even more common among people living with HIV. The risk of vertical transmission during delivery is about 50%. HIV-infected pregnant women with CT, NG, and/or syphilis infections are at increased risk of MTCT of HIV. Other studies on CT and NG infections in neonates are over 20 years old and those infections are not systematically measured in sub-Saharan Africa and are largely unknown.

Few countries provide routine antenatal testing for CT and NG. In most countries, including Botswana, the syndromic approach is used, which utilizes an algorithm to classify symptoms into STI syndromes and provide standardized treatment. Syndromic management lacks specificity and causes pregnant women to be unnecessarily exposed to antibiotics, and has low sensitivity, missing asymptomatic infections, putting neonates at risk. A study in Botswana found that among women with confirmed CT, NG, *Trichomonas vaginalis* (TV), only 10% reported vaginal discharge and 8% reported lower abdominal pain. In our pilot study, we found that less than 40% of women with etiologically diagnosed CT, NG, and TV infections reported having vaginal discharge, lower abdominal pain, and/or

painful urination. Thus, it's possible that many infections are being missed among pregnant women in Botswana. While testing for syphilis is a routine part of antenatal care in Botswana, previous studies have shown that testing coverage and receipt of treatment are suboptimal.

Our proposal has four specific aims. **Aim 1**: We will determine the burden of CT and NG infections and correlates of infection among asymptomatic pregnant women in Gaborone, Botswana by a) using diagnostic testing to estimate the prevalence of CT and NG infections at three time points: 1) first antenatal care visit, 2) third trimester antenatal visit, and 3) postnatal visit. We will also estimate the incidence of CT/NG infections between those visits and b) assess the correlates of infection. **Aim 2**: Will compare longitudinal neonatal outcomes for pregnant women tested for CT and NG infections during antenatal care with women who received standard antenatal care by a) estimating the frequency of vertical transmission of CT and NG infections and neonatal outcomes and the association with testing and treatment; and b) assessing independent factors that may be predictive of adverse neonatal outcomes. **Aim 3:** We will assess the burden of infection and markers of inflammatory response to CT and NG infection during pregnancy and associations with vertical transmission of CT and NG by a) testing immunologic factors associated with vertical transmission of CT and NG; b) determining the association between the Xpert® CT/NG assay's pathogen-specific cycle threshold value (Ct) and transmission; and c) evaluating the frequency and distribution of Sample Adequacy Control (SAC) cycle threshold values (Ct) from the Xpert® CT/NG assay, and evaluate any correlations with the transmission of CT/NG to neonates. **Aim 4:** We will determine the acceptability, feasibility, and costs of implementing point-of-care (PoC) syphilis testing and treatment among pregnant women in Gaborone, Botswana by offering: 1) dual HIV and syphilis PoC testing for pregnant women who are HIV uninfected/undiagnosed and 2) PoC syphilis testing for pregnant women with a known HIV infection with same-day treatment.

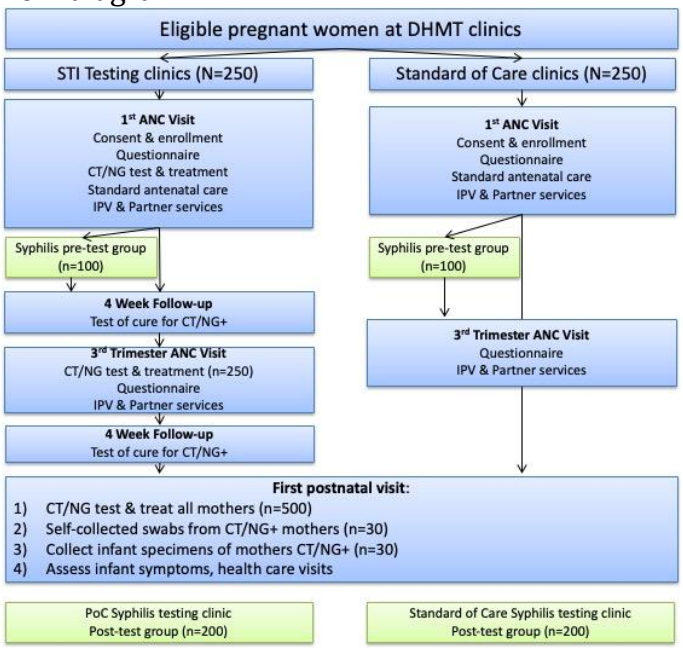
We will provide evidence to help evaluate the effects of testing on vertical CT/NG transmission and clinically important neonatal health outcomes, and to evaluate and understand biological correlates of CT/NG transmission.

2. **Specific Aims:**
    a. **Specific Aim 1:** To determine the burden of CT, NG infections and correlates of infection among *asymptomatic* pregnant women in Gaborone, Botswana.
        i. 1(a): Use diagnostic testing to estimate the prevalence of CT/NG infections at the 1st antenatal care visit, a 3rd trimester visit, and the 1st postnatal care visit; and estimate the incidence of infections between those visits.
        ii. 1(b): To assess the correlates of infection, including sociodemographic and behavioral factors (e.g. alcohol use), obstetric and medical information (e.g. HIV status), and sex partner characteristics.
    b. **Specific Aim 2:** To compare longitudinal neonatal outcomes for pregnant women tested & treated for CT/NG infections during antenatal care with women who received standard antenatal care.
        i. 2(a): To estimate the frequency of vertical transmission of CT/NG infections and subsequent neonatal outcomes (e.g. ophthalmia neonatorum) and the association with maternal testing and treatment during antenatal care.
        ii. 2(b): To assess independent factors that may be predictive of adverse neonatal outcomes and control for these factors in the analysis. Exposure measures will include maternal sociodemographic and behavioral factors (e.g. alcohol use), obstetric and medical information (e.g. HIV status), partner characteristics, and adverse perinatal outcomes (e.g. low birth weight).

c. **Specific Aim 3:** To assess the burden of infection and markers of inflammatory response to CT/NG infection during pregnancy and associations with vertical transmission of CT/NG.
   i. 3(a): To test immunologic factors associated with vertical transmission of CT/NG by describing concentrations of genital inflammatory cytokines in participants who test positive for CT/NG.
   ii. 3(b): To determine the association between the Xpert® CT/NG assay's pathogen-specific cycle threshold value (Ct), a possible marker for burden of infection, and vertical transmission of CT/NG to neonates.
   iii. 3©: To evaluate the frequency and distribution of Sample Adequacy Control (SAC) cycle threshold values (Ct) from the Xpert® CT/NG assay, which are a measure of tissue inflammation, and evaluate any correlations with the transmission of CT/NG to neonates.

d. **Specific Aim 4:** To determine the acceptability, feasibility, and costs of implementing point-of-care (PoC) syphilis testing and treatment among pregnant women in Gaborone, Botswana.
   i. 4(a) To assess the acceptability and feasibility of PoC syphilis testing and treatment. (Dual HIV and syphilis PoC testing will be offered to pregnant women who are HIV uninfected/undiagnosed and rapid syphilis testing for pregnant women with a known HIV infection). Our primary outcome will be defined as acceptability of testing, which is the proportion of women tested out of all those eligible and seeking care in the study clinics. Secondarily, we will assess the proportion of women who received treatment among those with a reactive syphilis test result. We will assess any treatment, treatment completion, and time to treatment.

Flow diagram



   ii. To evaluate the unit costs and patient costs associated with antenatal PoC syphilis testing and treatment compared to the standard of care the standard of care.

3. Study Design

Specific Aim 1: To determine the burden of CT and NG infections and correlates of infection among asymptomatic pregnant women in Gaborone, Botswana.

Methods and Procedures: To accomplish Aim 1, we will conduct a cohort study among antenatal patients at collaborating Greater Gaborone District Health Management Team (DHMT) clinic sites (STI testing clinics). First, 1(a): Use diagnostic testing to estimate the prevalence of CT/NG infections at the 1st antenatal care visit, a 3rd trimester visit, and the 1st postnatal care visit; and estimate the incidence of infections between visits. 1(b): To assess the correlates of infection, including sociodemographic and behavioral factors (e.g. alcohol use), obstetric and medical information (e.g. HIV status), sex partner characteristics, and partner notification and treatment outcomes.

Recruitment and Eligibility: We will recruit 250 study participants from pregnant women presenting for antenatal care services at designated study 'testing' clinics in Gaborone. Eligibility/Inclusion criteria (also described in Section E) include: 1) Age ≥ 15 years, 2) Currently pregnant, 3) Attending first ANC visit, 4) 27 weeks of gestation or less, 5) Not currently experiencing CT/NG-related symptoms (determined by validated screening tool), 6) Not treated for CT/NG in the past 30 days 7) Willingness to provide self-collected specimens for CT/NG testing (at three time points), 8) Willingness to return for a test of cure (if test positive during antenatal care), 9) Will reside in Gaborone through the time of delivery and 1st postnatal visit, 10) Willingness to have neonates tested for CT/NG at their first post-natal visit, And 11) Mentally competent to understand the informed consent.

Patients will be screened for eligibility via obstetric chart review at the time of the appointment. Those who are ineligible due to STI-related symptoms will be flagged and identified for healthcare providers to assess for syndromic management (standard care). All eligible patients will be provided information about CT and NG infections and study risks and benefits. Those providing informed consent will be enrolled, instructed on how to self-collect a CT/NG specimen and asked to share several forms of detailed contact information to assure follow-up. Reasons for ineligibility will be logged with age, gestational age, and reason for ineligibility, to assess generalizability. Similar data and reasons for decline will also be recorded.

A sample of up to 20 sex partners will be recruited for participation in a brief semi-structured interview. Partners will be recruited through the following channels a) snowball recruitment via study participants who give informed consent to contact their partner(s) b) sex partners presenting to a study clinic for STI treatment using a contact slip c) sex partners contacting the study team via contact information provided with EPT materials d) partners attending antenatal or postnatal care visits with partners. Eligibility criteria include 1) Age ≥ 15 years 2) Sex partner of a pregnant women being treated for CT, NG, Syphilis, or an STI syndrome 3) Willing to complete a recorded semi-structured interview and 4) Mentally competent to understand the informed consent.

A sample of up to 20 stakeholders will be recruited for participation in a brief semi-structured interview. Eligibility criteria include 1) a stakeholder, defined as a clinician who provides antenatal or sexual and reproductive healthcare, OR a program manager at a clinic that provides sexual and reproductive healthcare, OR a policymaker in the field of maternal and sexual and reproductive health and 2) Willing to complete a recorded semi-structured interview.

Specimen Collection, Transport, Processing and Storage: Specimens will be labeled with a unique study ID by trained study staff, who will place them in secure storage for up to 24 hours at 2° - 30°C until testing.

Laboratory Testing: CT /NG testing: will be performed using Xpert® CT/NG assay [Cepheid, Sunnyvale, CA] by trained staff. All specimens will be run on-site with standard controls, per manufacturer's instructions. (See Aim 3 for quality control). (Note: The Xpert is currently being used in Botswana for TB testing).

Reporting and Treatment: Antenatal CT and NG testing will be conducted at enrollment (1st ANC), during the third trimester, and at the 1st postnatal visit (See Figure 1). Results and treatment will be provided in the same way at each time period. Results: All participants will receive results on the same day as testing (in person or by phone). Treatment: Appropriate clinical staff will provide treatment per the Botswana MoH STI treatment protocols. Treated participants for CT/NG will be asked to provide a second specimen at least 4 weeks after treatment to document treatment outcome (test of cure). Test results will be recorded by study staff in the patients' treatment record files as well as into the study dataform. Partner Services: All study staff will be trained on appropriate and effective methods for counselling women on how to communicate test results to partner(s) in a nonthreatening and blameless manner. (For example, it's important to clarify that the duration of sexually transmitted infections during pregnancy is unknown, many infections are asymptomatic, and infections do not have implications about partner fidelity). Additionally, participants will be given several options for partner services: 1) No disclosure: In the case of risk for intimate partner violence (IPV). 2) Supportive disclosure: where the participant is assisted by trained study staff either in person or over the phone in notifying their partners. Also, participants will be given the option of bringing partners to the study clinics for counselling and treatment. 3) Expedited partner therapy for CT/NG treatment: where the participant can bring treatment to partners prior to examination by a healthcare provider. IPV: will be proactively assessed and managed for all participants, not only those testing STI positive. To that end, we will use a validated screening tool adapted from Rabin RF et al.[1] to assess participant risk for intimate violence and other negative partner responses to notification of an STI. Based on the screening results, when appropriate, IPV risk will be mitigated by referring women to local support services available in the country for free counseling and support, the NGO Botswana Family Welfare Association (BOFWA; http://www.bofwa.org.bw/). We will also provide a 24-hour emergency contact line through which participants can receive information and support and referrals in general.

Data collection: Participants will be asked to respond to a survey on socioeconomic characteristics, relationship status and sexual history (e.g. number of condomless sex acts), other risk behaviors (e.g. alcohol use), obstetric/medical history, and prior STI symptoms and treatment in the past year. A sub-set of participants will be asked additional questions on partner notification and treatment experiences postnatally. A data collection instrument will be used to abstract obstetric, and medical information from the patient-held and clinic records, including receipt of syphilis testing, results and titre, and treatment.

The sample of sex partners will participate in a brief semi-structured interview on partner notification and treatment experiences. The sample of stakeholders will participate in a brief semi-structured interview on antenatal diagnostic testing for chlamydia and gonorrhea, point-of-care syphilis testing, and STI expedited partner therapy.

Specific Aim 2: To compare longitudinal neonatal outcomes for pregnant women tested & treated for CT/NG infections with women who received standard antenatal care.

Methods and Procedures: Simultaneously with Aim 1, 250 antenatal patients at designated study 'standard of care' clinics will be enrolled as a comparison group receiving the standard care with additional partner services. At standard of care clinics, CT/NG testing will be offered to mothers and neonates at the 1st postnatal visit for all 500 participants to accomplish 2(a): To estimate the frequency of vertical transmission of CT/NG infections and adverse neonatal outcomes and association with maternal testing and treatment. And subaim 2(b): To assess independent factors associated with adverse neonatal outcomes and control for these factors in the analysis. Exposure measures will include maternal sociodemographic, behavioral, obstetric, and medical information, and partner treatment, and adverse perinatal outcomes (e.g. low birth weight).

Recruitment/Eligibility: Women will be similarly recruited and enrolled, and eligibility criteria are identical to those in the STI testing group (see Aim 1). Those who are ineligible due to STI-related symptoms will be flagged and identified for healthcare providers to assess for syndromic management (standard care). All eligible patients will be invited to participate, and those providing informed consent will be enrolled. The comparison group will receive the standard of care for management of STIs during antenatal care with additional IPV screening and partner services offered to those in Aim 1. As in Aim 1, a data collection instrument will be used to collect sociodemographic and behavioral factors, and obstetric/medical information, and data will be abstracted from the patient-held and clinic records. All mothers will be provided with informed consent for their neonate's participation at enrollment. Postdelivery, study staff will provide an updated verbal consent to mothers to collect and tests neonatal specimens.

Laboratory testing: All participants will test for CT/NG infection using the Xpert® CT/NG assay at the first postnatal visit using self-collected swabs. Women testing positive will receive antimicrobial treatment with options for partner treatment described in Aim 1. For neonates of mothers testing positive for CT/NG, the study follow-up nurse will collect ocular and nasopharyngeal swab samples for testing of CT/NG transmission using the Xpert® CT/NG assay. Studies have demonstrated the efficacy of PCR tests for infant diagnosis using ocular and nasopharyngeal swabs.[2, 3] Infants testing positive will receive treatment according to Botswana guidelines (20mg/kg of azithromycin (liquid formulation) for CT, injection of 50mg/kg of ceftriaxone for NG) and referrals.[4] Efforts will be made to provide same-day results.

1st Postnatal Visit Survey : Primary outcome will be collected by the study follow-up nurse and include: CT/NG test result from neonate ocular/nasopharyngeal swabs. Secondary outcomes include clinical evidence or records of conjunctivitis and history of pneumonia identified in interviews or medical charts; and inpatient length of stay. Data on potential confounders will be collected from three sources: 1) Interviewer-administered survey, AND 2) Patient obstetric record, OR 3) Hospital/clinic records. (Study staff have experience with all 3 sources). If new neonatal conditions are identified, the nurse will ensure necessary referrals.

Retention and Follow -up: Multiple forms of contact information will be collected at enrollment for all participants to ensure retention. All study visits will take place during regularly scheduled antenatal and postnatal care visits. Participants will be requested to give permission for communication through text messages, which will remind about upcoming visits. Participants who do not return for scheduled visits will be actively contacted by both clinic and study staff and encouraged to return for care. The study team will follow-up with all participants weekly around the expected date of delivery and participants will be encouraged to notify the team of first postnatal visit. Ten attempts will be made through various contact methods.

Specific Aim 3: To assess markers of inflammatory response to CT/NG infection during pregnancy and associations with vertical transmission of CT/NG.

Methods and Procedures: At the baseline, third trimester, and 1st postnatal care visit, self-collected vaginal swabs[5] and EDTA plasma samples will be collected from all participants in the STI testing group, and used to measure cytokine concentrations. Cytokines will be identified using the Luminex xMAP® 48-plex assay method located at Stanford University in order to accomplish 3(a): To test immunologic factors associated with vertical CT/NG transmission by describing concentrations of inflammatory cytokines in participants who tested positive for CT/NG. A secondary analysis of data collected during maternal CT/NG testing at the first postnatal visit (Aim 2) will be performed to accomplish 3(b): To determine the association between the Xpert® CT/NG assay's pathogen-specific cycle threshold value (C_t), which is a marker for the burden of infection, and vertical transmission of

CT/NG to neonates; and 3(c): To assess the frequency and distribution of Sample Adequacy Control (SAC) cycle threshold (C_t) values from the Xpert® CT/NG assay, which may be a marker of inflammation, and evaluate correlations with the transmission of CT/NG to neonates.

Recruitment/Eligibility: Eligibility criteria for the comparison group are identical to those in Aims 1 and 2. Data will only be collected from participants in the STI testing group.

Specimen Collection, Transport, Processing and Storage: A self-collected swab for cytokine measurement will be collected at the baseline, third trimester, and first postnatal care visit and stored at -80°C. A peripheral blood sample (2-4mL) will be collected at the baseline, third trimester, and postnatal study visits. The blood sample will be processed and aliquots of EDTA plasma will be stored at -80°C. The swabs and plasma samples will be transported in dry ice to Stanford University for processing. Luminex will be used to measure the concentrations of 48 cytokines in the 48-plex assay (including IL-1α, IL-1β, IL-6, IL-7, IL-8, IL-10, granulocyte macrophage colony- stimulating factor [GM-CSF], interferon-γ inducible protein [IP]-10, monocyte chemoattractant protein [MCP]-1, MIP-1α, MIP-1β, and tumor necrosis factor [TNF]-α). These cytokines were selected based on previously identified associations with genital infection.[6] Additionally the pathogen-specific and SAC Ct results will be automatically generated for each self-collected vaginal sample tested using the Xpert® CT/NG assay at delivery.

Laboratory Testing : Once the specimen is collected, study staff will follow instructions for sample preparation and testing. Luminex assays enable fast and accurate cytokine measurements by utilizing specially prepared microspheres, internally dyed with a graded mixture of red or infrared fluorescent dyes. Varying the degree to which the beads are internally dyed creates hundreds of different fluorescent profiles that can be individually interrogated and classified in a single sample.[7] The BHP lab in Gaborone can conduct cytokine analysis using an ELISA quantitative; however, this assay is currently limited to only IL-6, ICAM1, and VCAM. Further, the ELISA is a single assay and sample processing would be costly and time-consuming compared to the Luminex multiplex assay.

Specific Aim 4: To determine the acceptability, feasibility, and costs of implementing point-of-care (PoC) syphilis testing and treatment among pregnant women in Botswana by offering: 1) dual HIV and syphilis PoC testing for pregnant women who are HIV uninfected/undiagnosed and 2) PoC syphilis testing for pregnant women with a known HIV infection with same-day treatment to women in the designated PoC clinic. PoC testing will be offered in addition to routine syphilis and HIV testing.

Methods and Procedures: An additional 200 antenatal patients at the designated PoC clinic and 200 at the 'standard of care' clinic will be enrolled (400 total). In the PoC clinics, participants who are HIV uninfected/ undiagnosed will be offered PoC dual HIV/syphilis testing and women living with HIV will be offered a PoC syphilis test. PoC participants will receive same-day results and treatment. Participants in the standard of care clinic will continue to receive the usual care.

Additionally, analysis will incorporate the data from 200 participants previously enrolled in the CT/NG parent study. These data will be used as a "pre-test" and will include all 100 women enrolled in the standard of care clinic and all 100 women enrolled in the PoC testing clinic.

Recruitment and Eligibility: Recruitment will be the same as Aim 1 and the risks and benefits of PoC syphilis testing will be explained. Reasons for declining to participate will be recorded as well as basic information, including age and gestational age to assess generalizability. Eligibility criteria will be the same as Aim 1, after removing CT/NG testing-specific criteria, and will include: 1) Age ≥ 15 years, 2) Currently pregnant, 3) Attending first ANC visit, 4) 27 weeks of gestation or less, 5) Will reside in

Gaborone through the time of delivery and 1st postnatal visit, And 6) Mentally competent to understand the informed consent.

Laboratory Testing: PoC syphilis: Participants who are HIV-uninfected/undiagnosed in the PoC clinics will receive a test using the SD BIOLINE HIV/Syphilis Duo test, which is a solid phase immunochromatographic assay for the qualitative detection of antibodies to all isotypes(IgG, IgM, and IgA) specific to HIV-1/2 and/or Treponema pallidum (TP) simultaneously in human serum, plasma, or whole blood. The WHO prequalification performance evaluation found a final sensitivity for HIV was 100% (95% CI: 98.2-100%) and specificity of 99.5% (95% CI: 97.2-100%). The final sensitivity for antibodies to *treponema pallidum* was 87% (95% CI: 81.5%-91.3%) and the specificity was 87% (95% CI: 97.2-100%).[8] As per Botswana guidelines, HIV will be diagnosed with two serial HIV reactive test results (Dual PoC followed by rapid HIV test). For participants who are living with HIV and seeking care in the PoC clinics, we will offer a rapid, PoC blood test for syphilis with WHO prequalification. All participants will continue to receive the standard of care, non-treponemal detecting IVD (RPR/VDRL) test. Results from the RPR/VDRL will be used to confirm test results and differentiate individuals with active (untreated) infection from those who have been successfully treated for past infection.[9]
Reporting and Treatment: PoC Results: All participants will receive results in person, on the same day as testing. Treatment: Appropriate clinical staff will provide treatment per the Botswana MoH STI treatment protocols (see Treatment section below). Test results will be recorded in the participant obstetric record, clinic records, as well as into the study data form. Partner Services will be offered in the same manner as Aim 1; however, expedited partner treatment will not be an option for syphilis treatment. Standard of Care: Participants will continue to receive syphilis testing per Botswana national guidelines, which includes a VDRL/RPR test at the first ANC visit with delayed results and treatment. Typically, blood specimens are sent to a central lab for processing and results are provided to clinic staff after at least three days. Women are called to return to the clinic for receipt of results. Test results and treatment are recorded in the patient-held and clinic records.

Data collection: For participants in the PoC clinic, acceptability of PoC syphilis testing and receipt of treatment (with dates) will be recorded by study staff in study documents, and the patient-held and clinic records. Participants who decline and reasons for decline will be recorded in study records. For participants in the standard of care clinic, the patient-held and clinic obstetric records will be reviewed to identify receipt and dates of syphilis testing and treatment. We will also abstract obstetric and health information (para, gravida, gestational age, HIV status, antiretroviral treatment) from the records of all participants. At the time of enrollment, participants will be asked to respond to a short interviewer-administered questionnaire to collect basic sociodemographic information and costs incurred related to syphilis testing. Participants will also respond to another short survey on costs during their third trimester, which will be conducted over the phone. Cost-related questions will inquire about travel, overnight lodging, out-of-pocket care costs; and the opportunity costs associated with travel, wait, and appointment times. Participants testing positive will be eligible to participate in a brief semi-structured interview on partner notification and treatment.

Outcomes: Our primary outcome is acceptability of syphilis testing at the first ANC visit (proportion tested for syphilis out of all eligible women receiving care in the study clinics during implementation of the study). For participants in the PoC clinic, acceptability will be defined as having a PoC test with recorded results. Those who decline PoC testing will be identified as not accepting and will be included in the denominator. For those in the standard of care, the numerator will be women who receive an RPR/VDRL syphilis test recorded in patient-held or clinic records. The denominator for both groups will be all women attending the study clinic during implementation and eligible for participation (1. Age ≥ 15 years, 2. Currently pregnant, 3. Attending first ANC visit, 4. 27 weeks of gestation or less, 5. Will reside

in Gaborone through the time of delivery and 1st postnatal visit, And 6. Mentally competent to understand the informed consent).

Additional outcomes will be related to treatment and costs. We will report the proportion of women who received treatment among those with a reactive syphilis test result. We will assess differences in any treatment, treatment completion, and time to treatment.

The primary cost outcome is the average unit cost per woman tested for each strategy and will include both patient-related costs and healthcare system costs. Healthcare-related cost data will be collected from in-country data sources. Patient data will be collected from the short survey.

Analysis: First, comparisons will be made across four groups of participant data: **1)** participants in the standard of care clinic enrolled as part of the CT/NG study prior to implementation of the syphilis testing sub-study called "Standard of Care pretest" (n=100); **2)** participants in the standard of care clinic enrolled as part of the syphilis testing sub-study called "Standard of Care post-test" (n=200); **3)** participants in the PoC clinic enrolled as part of the CT/NG study prior to implementation of the syphilis testing sub-study called "PoC pretest" (n=100); and **4)** participants in the PoC clinic enrolled as part of the syphilis testing sub-study called "PoC post-test" (n=200).

To assess differences in receipt of syphilis testing, we will use a difference-in-difference (DiD) strategy, which takes the difference between two before and after differences (pre-post-test with comparison).[25] DiD will be performed using a logistic regression model with a variable for whether the individual was in the PoC clinic (PoC), whether or not it is the post period (Post), an interaction variable for the product of those two variables, and covariates. The primary outcome (Y) is the proportion of eligible women who are tested for syphilis at their first ANC visit:
Y=a+bPoCPoC+bPostPost+bint(Post*PoC)+bcovariates Models will also be stratified by HIV status to see if PoC had a differential impact among WLH. Variables for which there were significant differences between the intervention and comparison group will be included in multivariable model.

Secondarily, will also assess receipt of treatment using DiD analysis by comparing the pre-test and post-test differences in terms of the proportions of participants who had a reactive test who: 1) received the first dose of treatment, and 2) completed treatment. Prevalence of syphilis will also be reported according to treatment group and HIV status as the proportion of individuals testing positive divided by the number of individuals tested. Exact binomial 95% confidence intervals for prevalence will be estimated. All analyses will be conducted with Stata 17.0 (Stata Corporation, College Station, TX).

To compare differences in costs, recurrent costs will be calculated per test, and capital and training costs will be summed and divided by the number of women tested. Those costs will be added to get an average unit cost for each strategy. For the intervention strategy, we will also calculate the average cost by HIV status at first ANC (i.e. the cost of delivering the dual HIV/syphilis test and the rapid syphilis). We will compare the mean costs using the non-parametric Mann-Whitney U test.[10] For hypothesis 4, patient costs will be summed and an average patient cost will be calculated for each strategy, which will be compared using either the Mann-Whitney U test or Student's t-test. Cost components will be examined to determine key cost drivers and to identify potential efficiencies that could be achieved in future scale-up.

4. Background and Significance

Chlamydia trachomatis (CT), *Neisseria gonorrhoeae* (NG) and syphilis (caused by *Treponema pallidum*) infections are bacterial STIs that cause adverse neonatal outcomes.[11-22] The risk of vertical

transmission of CT/NG during delivery is about 50%.[23] Among infants born to mothers with untreated CT infection, 30–50% develop clinical conjunctivitis and 10-20% develop pneumonia due to CT.[23, 24] Among infants born to mothers with untreated NG infection, the risk of conjunctival infection is up to 48%, which can result in corneal damage and blindness.[25] A recent study in South Africa found that among infants born to a mother with CT, NG, or *Trichomonas vaginalis* (TV) infections, 37% had nasopharyngeal colonization with the same STI organism.[26] Other studies on CT/NG infections in neonates are over 20 years old.[25, 27, 28] HIV-infected pregnant women with CT/NG infections are at increased risk of MTCT of HIV.[29, 30] Untreated syphilis during pregnancy is associated with increased risk for early fetal death, stillbirth, preterm birth, low birthweight, neonatal death, and clinical disease in infants.[22] Congenital syphilis is the second leading cause of preventable stillbirth globally.[31]

According to the WHO, CT, NG, and syphilis are the most common STIs globally with 131 million new cases of CT, 78 million new cases of NG infections, and 6 million new cases of syphilis annually.[32] These infections are more common among people living with HIV.[33] Despite the large burden of CT/NG infections and the above mentioned adverse outcomes, few countries have guidelines that recommend routine antenatal CT/NG testing.[34] The WHO STI Treatment Guidelines are silent on the issue due to lack of evidence.[35] In most low/middle countries, syndromic management (SM) is used to manage CT/NG infections. SM utilizes an algorithm to classify symptoms and clinical signs into STI syndromes, and patients are treated with standardized drug regimens.[35, 36] SM lacks specificity, causing unnecessary exposure to antibiotics, and has low sensitivity, missing the large proportion of asymptomatic infections and potentially putting neonates at risk for transmission.[37-40] A study in Botswana found that among women with confirmed CT, NG, *Trichomonas vaginalis* (TV), only 10% reported vaginal discharge and 8% reported lower abdominal pain.[41] In our pilot study, we found that less than 40% of women with etiologically diagnosed CT, NG, and TV infections reported having vaginal discharge, lower abdominal pain, and/or painful urination.[33] Thus, it's possible that many infections are being missed among pregnant women in Botswana. Botswana national guidelines recommend universal screening and treatment of syphilis during pregnancy, which is conducted using the rapid plasma reagin (RPR) test or the venereal disease research laboratory (VDRL) with delayed results and treatment. However, a national surveillance study reported that only 59% of those with a reactive RPR were treated,[42] and treatment was delayed by five weeks on average.[43]

As accurate and rapid testing technology is increasingly affordable, more evidence on the benefits of antenatal CT/NG testing is needed. This study will utilize an innovative reverse transcription polymerase chain reaction (PCR) assay that can provide results within 90 minutes. Xpert® CT/NG also has high sensitivity (97.6% and 95.6% for CT/NG respectively) and specificity (99.4% and 99.8% for CT/NG respectively) and functions well in resource-constrained environments such as those proposed here.[44] For syphilis testing, we will use WHO pre-qualified dual syphilis/HIV point-of-tests and rapid syphilis tests (SD Bioline), which will be offered in addition to the standard of care syphilis screening procedure.

The proposed study will estimate the prevalence and correlates of infection among pregnant women who present to antenatal care without STI-related symptoms, and thus would not be treated for infections through the standard care. We will assess the impact of antenatal CT/NG testing to prevent vertical transmission and associated adverse health outcomes compared to the standard of care. We will use markers of tissue inflammation specific to the Xpert® to explore the inflammatory responses to CT/NG and associations with vertical transmission. We will assess the acceptability, feasibility, and costs associated with PoC dual syphilis/HIV testing. We hope to inform stakeholders and policy makers as they work to improve management of STIs during antenatal care in order to improve maternal and neonatal outcomes.

We will include women aged 15-17 years, who are pregnant and receiving care, because pregnancy and curable sexually transmitted infections (STIs), such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and syphilis are common among adolescents in Botswana. Recent research by BHP determined that of 737 adolescent females, aged 16-19 years, 340 (46%) reported being sexually

active. Of these, 121 (36%) reported having at least one child. A 2007 study of STIs among pregnant women in Gaborone, found that age was a strong predictor of *C. trachomatis* and/or *N. gonorrhoeae* infections; and, in a sample of 703 pregnant women, the prevalence of cervical infections was highest among teenagers (22%; 95% CI: 13-32%).[41] Recent research in Southern Africa has similarly found that the prevalence of STIs was higher among younger women.[45] Thus, in order to understand the burden and address the increased risks for STIs faced by women under 18 years, more research is needed among this group.

5. Characteristics of the Subject Population:

a. Number of subjects / Sample size:
Pregnant women attending their first ANC care visit at 27 weeks gestation or less. Participants must be 15 years of age and not treated with azithromycin, doxycycline, cefixime, or ceftriaxone in the past 30 days.

Specific Aim 1 (STI testing group): We will recruit 250 study participants for CT and NG testing.

Specific Aim 2 (Standard of care comparison group): We will recruit 250 antenatal patients from designated study 'standard of care' clinics who will be enrolled as a comparison group receiving the standard care with additional IPV and partner services and postnatal care testing (Total N=500). Among participants who test positive during postnatal care, we will offer infant testing (Estimated N=30 infants).

For Specific Aims 1 & 2, study staff will remain at study clinics during operating hours for the duration of the recruitment period. We will consecutively screen all pregnant women for eligibility and offer enrollment to all eligible women.

Specific Aim 3 (Inflammatory markers): We will include women in the STI testing group (Estimated N=250).

Specific Aim 4: We will enroll an additional 400 antenatal patients for the PoC syphilis sub-study (200 will receive the standard of care and 200 will be offered PoC syphilis testing in addition to the standard of care)

b. Inclusion/Exclusion Criteria:

- **Inclusion Criteria:**
    - Age ≥ 15 years,
    - Currently pregnant,
    - Attending first ANC visit,
    - 27 weeks gestation or less
    - Not currently experiencing CT/NG-related symptoms (determined by validated screening tool),
    - Not treated for CT/NG in the past 30 days,
    - Willingness to provide self-collected specimens for CT/NG testing (for the STI-testing group, this will take place at their first ANC visit, at another visit in their third trimester,

and at postnatal care. For the standard of care group, samples will only be collected at postnatal care),
- Willingness to return for a test of cure if CT/NG test is positive during antenatal care,
- Will reside in Gaborone through the time of delivery and 1st postnatal visit,
- Willingness to have neonates tested for CT/NG at their first postnatal visit,
- Mentally competent to understand the informed consent.

- **Exclusion Criteria:**
  - Not mentally competent to understand study procedures or give informed consent,
  - Individuals < 15 years,
  - Men,
  - Women who are not pregnant,
  - Pregnant women not attending their first antenatal visit,
  - Pregnant women at >27 weeks gestation
  - Pregnant women with current STI-related symptoms (will receive standard of care),
  - Treated for an STI in the past 30 days

c. Determining Eligibility --- Study Screening: Screening of prospective participants is confidential and will be undertaken by study personnel with training in ethical treatment of human subjects.

d. Reasons for Exclusion:
This study is designed to investigate benefits to women and infants of testing asymptomatic pregnant women for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* during their first and third antenatal care visit. Therefore, women who are not pregnant as well as men are excluded from participation. Women with symptoms will receive syndromic management and those treated in the last 30 days are likely to be cured of STIs.

e. Vulnerable Subjects: As this study is conducted in Botswana, all interviews and exchange of information is conducted in English or Setswana. As the study concerns antenatal and postnatal testing, participants are pregnant women and infants.

f. **Inclusion of women aged 15-17 years**: As we are including participants under 18 years, we will add additional protections for minors such as: reporting requirements for abuse and referrals to social workers and added support for partner notification. We have also added an adolescent health expert to our team to advise on these issues.

6. Subject Identification and Recruitment:

Specific Aim 1: Patients will be screened for eligibility via obstetric chart review and brief screening questions at the time of the appointment. All eligible patients will be provided information about CT/NG infections and study risks and benefits. Those providing informed consent will be enrolled, instructed on how to self-collect a CT/NG specimen and asked to share several forms of detailed contact information to assure follow-up. Reasons for ineligibility will be logged with age, gestational age, and reason for ineligibility, to assess generalizability. Similar data and reasons for decline will also be recorded. A data collection instrument will be used to collect sociodemographic, behavioral, sex partner, obstetric, and medical information. Data will be abstracted both from patient medical records and interviews by study staff.

The sample of sex partners will be recruited through the following channels a) snowball recruitment via study participants who give informed consent to contact their partner(s) b) sex partners presenting

to a study clinic for STI treatment using a contact slip c) sex partners contacting the study team via contact information included with EPT materials (with permission of index patient) d) partners attending antenatal or postnatal care visits with partners.

The sample of stakeholders will be recruited through email, word of mouth and visits to study clinics.

Specific Aim 2: Women in the Standard of Care group will be similarly recruited and enrolled, and eligibility criteria are identical to those in the STI testing group (see Aim 1). All eligible patients will be invited to participate, and those providing informed consent will be enrolled. The Standard of Care group will receive the standard of care in Botswana for the management of STIs with additional intimate partner violence (IPV) screening and partner services offered to those in Aim 1. As in Aim 1, a data collection instrument will be used to collect sociodemographic and behavioral factors, and obstetric/medical information. All mothers will be provided with informed consent for their neonate's participation at enrollment. Postdelivery, study staff will provide an updated verbal consent to mothers to collect and tests neonatal specimens.

Specific Aim 3 At the baseline, third trimester and postnatal care study visit, self- collected swabs [5] and plasma samples (2-4mL) will be collected from participants in the STI testing group, and used to measure cytokine concentrations. Cytokines will be identified using the Luminex xMAP® 48-plex assay method located at Stanford University in order to test immunologic factors associated with vertical CT/NG transmission by describing concentrations of inflammatory cytokines in participants who tested positive for CT/NG. We will also conduct a secondary analysis of data collected during maternal CT/NG testing at the first postnatal visit (Aim 2) will be performed to determine the association between the Xpert® CT/NG assay's pathogen-specific cycle threshold value (C_t), which is a marker for the burden of infection, and vertical transmission of CT/NG to neonates; and assess the frequency and distribution of Sample Adequacy Control (SAC) cycle threshold (C_t) values from the Xpert® CT/NG assay, which may be a marker of inflammation, and evaluate correlations with the transmission of CT/NG to neonates.

Specific Aim 4: Women in the syphilis sub-study will be similarly recruited and enrolled as in Aim 1 and 2. All participants will receive the standard of care in Botswana for syphilis testing with additional IPV screening and partner services offered to those in Aims 1 and 2. A simplified data collection instrument will be used to collect sociodemographic and behavioral factors, and obstetric/medical information with additional questions related to patient costs incurred due to syphilis care. Participants recruited into the PoC syphilis testing clinic will provide a blood sample for PoC syphilis testing and treatment.

7. **Methods and Procedures applied to human subjects**

a. **Study Protocol used by study personnel at Greater Gaborone DHMT Clinics:**

Enrolment, follow up visits and sample collection

- All participant recruitment will occur in up to eight DHMT clinics in Greater Gaborone. Study staff will be trained in the study's methods, protocol, and human subjects research, and will conduct eligibility screening on all patients attending their first ANC visit, following a simple, standard checklist of eligibility criteria. Staff will read all eligible women a brief description of the study. Interested women will then be read aloud the study consent form in their preferred language, which will provide specific information about sexually transmitted infections, the consequences and treatment of those infections, and study risks and benefits, and then will be invited to participate in the study. Staff will collect basic

de-identified information from clinic logs (i.e., age, gestational age, HIV status) to use for descriptive analysis of the general ANC patient population.

- Study personnel will inform potential participants about the risks and benefits of study participation and answer any questions they may have concerning the study protocol. Study personnel will then ask interested eligible women sign an informed consent for participation (consent form in appendix).

- To ensure retention, those providing informed consent will be asked to provide detailed contact information (e.g., phone numbers and "home address" for self, family, friend/neighbor) to ensure follow-up. To develop and maintain a strong relationship with participants, study staff will conduct welcome phone calls within 3 days of enrollment, and check in with participants during regular ANC clinic visits. Clinic and study staff will contact participants who do not return for scheduled ANC visits and encourage return for care. We will flag participant charts so that clinic staff will notify study staff on the date of delivery.

- During the antenatal visits (1st antenatal care and a visit in the 3rd trimester), participants in the STI testing group will be asked to provide self-administered vaginal swab samples for CT/NG testing by the GeneXpert and cytokine testing by Luminex. Participants will also be asked to provide additional samples that will be stored for testing of *Trichomonas vaginalis*, bacterial vaginosis, and/or *Mycoplasma genitalium*. These samples will be stored at the BHP lab for no longer than 24 months. Participants will also be asked to provide a plasma sample, collected by a trained phlebotomist, for cytokine testing by Luminex.

- During the first antenatal visit, a sub-sample of participants in the standard of care group will be asked to provide two self-administered vaginal swabs for testing after study completion. These samples will be immediately de-identified and stored at the BHP lab for no longer than 24 months.

- During the 1st antenatal care visit, participants in the syphilis PoC clinic will be asked to provide a blood sample for either dual HIV/syphilis or rapid syphilis point-of-care testing, which is offered in addition to the standard of care.

- During postnatal care, all participants will self-collect a vaginal sample for CT/NG testing by the GeneXpert. Participants in the testing group will self-collect an additional vaginal sample and will provide a plasma sample for cytokine analysis. Those who test positive will be offered infant testing via ocular/nasopharyngeal samples. Infant samples will be collected by a trained study nurse. Participants will also be asked to provide additional samples at the postnatal visit that will be stored for testing of *Trichomonas vaginalis*, bacterial vaginosis, and/or *Mycoplasma genitalium*. These samples will be stored at the BHP lab for no longer than 24 months.

- Postnatally, a sub-set of 35 participants who were treated for CT/NG, an STI syndrome or syphilis at any point during the study will be asked additional questions by study staff, expanding on partner

notification and treatment experiences. These patients will additionally be asked if they are willing to refer their partner to participate in a semi-structured interview. If so, they will sign an informed consent allowing the study team to contact their partner(s).

- Specimens for CT/NG and syphilis testing will be processed onsite at the antenatal clinics by study personnel.

- Specimens for *Trichomonas vaginalis*, bacterial vaginosis, and/or *Mycoplasma genitalium testing* will be stored for up to 24 months at BHP.

- The self-collected swabs and EDTA plasma aliquots for cytokine measurement will be stored at -80 C. The swabs and EDTA plasma will be transported in dry ice to Stanford University for processing. Luminex will be used to measure the concentrations of 48 cytokines (including IL-1a±, IL-1b≤, IL-6, IL-7, IL-8, IL-10, granulocyte macrophage colony- stimulating factor [GM-CSF], interferon-y≥ inducible protein [IP]-10, monocyte chemoattractant protein [MCP]-1, MIP-1a±, MIP-1b≤, and tumor necrosis factor [TNF]-a±). These cytokines were selected based on previously identified associations with genital infection.(34) Additionally the pathogen-specific and SAC Ct results will be automatically generated for each self-collected vaginal swab sample tested using the Xpert CT/NG assay at the first postnatal visit.

Results

- Results of the CT/NG testing will be available to the women after 90 minutes, placed in the medical record, and communicated to medical providers. Efforts will be made to provide results to participants on the same day.

- Results for syphilis will be available to women after 20 minutes, placed in the medical record, and communicated to medical providers. Efforts will be made to provide results to participants on the same day.

Treatment

- Women diagnosed with CT will receive a single dose of oral azithromycin (1 gram) and women diagnosed with NG will receive a single dose of oral azithromycin (1 gram) and a ceftriaxone 250 mg injection will be administered on site according to standard of care per Botswana MOH guidelines.

- Infants testing positive will receive treatment according to Botswana guidelines (20mg/kg of azithromycin (liquid formulation) for CT, injection of 50mg/kg of ceftriaxone for NG) and referrals

- Women testing positive for syphilis either through the dual syphilis/HIV or rapid syphilis test will be counseled according to Botswana guidelines, including assessing previous syphilis diagnoses and treatment and penicillin allergies. Treatment will also be initiated according to national guidelines. As Botswana is a low syphilis prevalence setting (<5%), the national guidelines follow WHO recommendations and currently recommend Benzathine penicillin 2.4MU IM weekly for 3 weeks with alternative treatments for pregnant women with penicillin allergies. For women with reactive

treponemal tests, the standard of care RPR test titre results will be provided to the participant's physician to inform further treatment/management.

Test of cure

- A test of cure will be performed approximately four weeks after initial treatment for CT/NG during an antenatal care visit to document clearance of infection. If patients are positive on repeat testing, they will be retreated and given additional counseling regarding CT and NG and additional efforts will be made to follow up, counsel and treat any new sex partners or old sex partners who have not been treated.

Partner Services

- All study staff will be trained on appropriate and effective methods for counselling women on how to communicate test results to partner(s) (from the past 60 days) in a nonthreatening and blameless manner. (For example, it's important to clarify that the duration of sexually transmitted infections during pregnancy is unknown, many infections are asymptomatic, and infections do not have implications about partner fidelity). Additionally, participants will be given several options for partner services: 1) No disclosure: In the case of risk for intimate partner violence (IPV). 2) Supportive disclosure: where the participant is assisted by trained study staff either in person or over the phone in notifying their partners. Also, participants will be given the option of bringing partners to the study clinics for counselling and treatment. 3) Expedited partner therapy: where the participant can bring CT/NG treatment to partners prior to examination by a healthcare provider. IPV: will be proactively assessed and managed for all participants, not only those testing STI positive. To that end, we will use a validated screening tool adapted from Rabin RF et al.[1] to assess participant risk for intimate violence and other negative partner responses to notification of an STI. Based on the screening results, when appropriate, IPV risk will be mitigated by referring women to local support services available in the country for free counseling and support, the NGO Botswana Family Welfare Association (BOFWA; http://www.bofwa.org.bw/). We will also provide a 24-hour emergency contact line through which participants can receive information and support and referrals in general.

- Efforts will be made to ensure that partners return to the study clinics for treatment. Those who return will receive the stat doses of azithromycin and ceftriaxone or Benzathine penicillin as described above for participants. In cases where participants and study staff are concerned about partner access to prompt clinical evaluation and treatment for CT/NG, partners will be offered expedited therapy (EPT) whereby participants will be provided with treatment to bring home for partner(s).[46] For CT, EPT will consist of a single dose of oral azithromycin (1 gram). For NG, EPT will consist of oral cefixime (400mg) and a single dose of oral azithromycin (1 gram).[47]

8. **Research involving survey, questionnaires:** There is a questionnaire for demographic data and medical history for participants that will be filled out by study staff and a brief semi-structured interview on partner notification and treatment experiences that will be conducted by study staff in a sub-set of participants.

9. **FDA approval (e.g. for experimental drugs, etc.)---- N/A**

Title: The diagnosis and treatment of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in pregnant women to prevent adverse neonatal consequences."

10. Data collection, Storage and Confidentiality:

Data will be collected electronically using REDCap. Data stored in REDCap will not have personal identifiers and personal identification codes will be used. A code sheet linking the data to the subject's personal identification will be stored separately from the REDCap data in a locked research office in the BHP offices. Only authorized study personnel will have access to the REDCap data and the linking form. Each staff member will have their own secret login and REDCap logins will be monitored by the study manager.

11. Risk/Benefit Assessment (12-17)

a. Potential Risks and Discomforts:
The potential risks encountered during the study are: 1) CT and NG testing 2) Specimen collection. 3) Questionnaire. 4) Data analysis

Conducted by Study Personnel at Princess Marina:
The primary potential risks to participants due to study participation are psychological and social in nature. Physical risks from a self-collected vaginal specimen are negligible. Unlike HIV and syphilis testing, screening for CT or NG is not a routine part of the standard of care for pregnant women in Botswana, participants in the comparison group have no risk greater than that normally incurred during a typical antenatal care visit; i.e. mild physical or psychological discomfort. IPV is a possible risk when women test positive for CT/NG. IPV will be proactively assessed and managed for all participants using a validated screening tool to assess participant risk for intimate violence and other negative partner responses to notification of an STI. Based on the screening results, when appropriate, IPV risk will be mitigated by referring women to local support services available in the country for free counseling and support. We will provide a 24-hour emergency contact line through which participants can receive information and support and referrals in general. Also, women will be given several options for partner notification and treatment.

A. Psychological: Participants could experience psychological distress such as anxiety when discussing issues related to personal experiences, sexual health, or pregnancy. However, we do not expect any serious events to occur based on our experience across multiple previous studies, including our pilot study with this same population in Botswana. Participants may experience some stress related to the knowledge of STI status. Participants will be given information and education about the nature and consequences of all infections and treatment, and those testing positive (including newborns) will be provided treatment as per standard treatment protocols. The likely harmful consequences of learning one's STI status are low.

B. Social: Participation in this study may cause social harm (e.g., discrimination, false assumptions, rumors) if the participation of the participant becomes known to others. One of the more significant risks is notification of sexual partners about positive results of CT or NG testing, which is an important step to protect the health of the partners and their future contacts. It is possible that notifying partners about a positive CT or NG test could put the participant at risk for IPV. Given this, we will provide IPV prevention counseling and will take steps to mitigate and monitor such outcomes, providing intensive participant support as needed.

C. Alternative treatments and procedures: Participants from the target population invited to participate will be able to decline participation, with no consequence to them.

b. Risk Classification: Minimal.

c. Minimizing Risks:
Informed consent and strict confidentiality will be rigorously enforced to minimize risks to participants and their partners. All study personnel will receive training in the informed consent process.

Additional protections will be put in place for participants under the age of 18 including reporting requirements for abuse and referrals to social workers and added support for partner notification. We have also added an adolescent health expert to our team to advise on adolescent-related issues.

Trained providers will be available in case participants have difficulty in any part of the specimen collection such as pain or discomfort or in the case of any adverse event.

Participants will be provided with a number whereby a member of the clinic staff and the Principal Investigator, Dr. Chelsea Morroni, may be contacted to answer questions or in case of emergency or any questions regarding their informed consent.

d. Potential Benefits:

Conducted by study personnel:
Diagnosis and treatment of CT/NG may prevent the development of future complications in reproductive health as well as transmission of undiagnosed infections to newborns and sexual partners, and reduce the risk of adverse birth outcomes. Diagnosis and treatment of CT/NG may address symptoms among infants. Participants in the syphilis PoC testing clinics will have access to rapid syphilis results, which may increase the likelihood of treatment access for those who test positive and may reduce the need for non-routine follow-up visits.

Potential benefits to society include determination of the benefits (in terms of infections cured and prevention of vertical transmission of STIs and subsequent adverse outcomes) of integration of routine CT/NG screening into antenatal care in Botswana. The study will also provide valuable operational data regarding of patient follow-up and treatment, as well as insights into the formation and revision of antenatal care policies, guidelines and funding levels. Results will serve to estimate prevalence of CT/NG infections among asymptomatic pregnant women in Gaborone, Botswana.

Risk/Benefit Ratio: The benefits to the participants in diagnosis and treatment of CT/NG infection outweigh the risks of physical or personal discomfort from specimen collection. As syphilis testing is already part of the standard of care, there are no additional testing risks. As described above, confidentiality of study data will be rigorously maintained. The benefits of PoC syphilis testing include same day results and treatment.

12. Therapeutic Alternatives: Participation in the study protocol is voluntary. Patients declining participation will still receive treatment from their physician, including STI screening, according to current standards of care.

13. Financial Considerations:

a. Payment for Participation: Participants will receive 50 Pula to help compensate for attendance at each study visit including baseline, test of cure, third trimester, postnatal, and infant testing visits.

b. Financial Obligations of the Subjects: There are no costs to participate in the study.

14. Emergency Care and Compensation for Research-Related Injury:

Minimal risk is associated with participation in this study. A telephone number is provided on the informed consent form for participants to speak with a study clinician if they believe they have experienced a research-related injury. Study personnel will help participants obtain appropriate care or direct them to the hospital's emergency room if they believe they have a life-threatening emergency.

15. Informed Consent:

a. Capacity to consent: All subjects will have the capacity to give informed consent.

b. Personnel Inviting Participants:
Study personnel will invite potential participants and will help them to understand the informed consent document. Only after all the information has been provided and the potential participants have all their questions about the study answered, will staff ask them to sign the informed consent form to participate in the study. The process of obtaining informed consent will be finished before collection of data or specimens. Study personnel will give a copy of the consent document to the participants to keep. They will exclude any potential participant who is unable to understand the informed consent, severely mentally disabled, or does not meet age criteria. They will keep consent forms in a locked office in Gaborone. All study staff involved in recruiting and consenting participants will have completed the Botswana equivalent of a Human Research Subjects certification program before the beginning of the study.

16. Process of Consent:

After verification of eligibility, the informed consent process is as follows:

- The study interviewer says: "We would like to read through this consent form with you because we want to make sure that you understand everything in it and that we answer any questions you may have." The study interviewer reviews the consent form, paragraph by paragraph, and stops as needed for any questions or clarifications.

- The interviewer asks the potential participant short questions about the consent to make sure that she fully understands the study. This process gives the interviewer time to assess the potential participant to make sure there are no "gross Impairments" that would invalidate the informed consent (e.g. participant does not fully understand the study, is intoxicated, etc.) and make study participation unethical and prohibited.

- If the potential participant agrees to participate in the study, the study interviewer then signs and dates the consent form. The participant's study code is then written on the consent form.

- The subject is given a copy of the consent form.

17. Comprehension of the Information Provided:

The study personnel will ask participants to restate in their own words the study's objectives and what participation involves to determine if they fully understand the informed consent. If the participant does not understand the protocol, the study interviewer will explain again the content of the consent form. If the participant is still unable to understand the protocol after it is reviewed again, she will be excluded from participation in the study.

18. **Information withheld from Subjects:** Not applicable.

19. Consent/Assent forms: The consent form used in the study is in the appendix.

20. **Conflicts of Interest**

The investigators involved in this study have no conflicts of interest to report.

21. Study Timeline

Table 1: Study Timeline	**Start**	**Finish**	**Duration**
Component and Task Name			
I. Preparation and Piloting	Jan 2020	Dec 2020	12 mo.
II. Enrollment and Follow-up (CT/NG)	Feb 2021	Mar 2022	11 mo.
III. Enrollment (syphilis post-test group)	August 2022	Feb 2023	7 mo.
IV. Follow-up only (CT/NG)	Apr 2022	Dec 2022	9 mo.
V. Data Analysis and Dissemination	Jan 2023	May 2023	5 mo.

22. Dissemination Plan:

Test results and educational information will be shared with patients. Results of the study will be compiled into a report and presented to the Ministry of Health. Results will also be presented at local and international scientific conferences. Additionally, the study will be described in an article that will be submitted for publication in a peer-reviewed journal and results will be presented at national and international meetings. Finally, the results of the study will be shared with patients and staff at Gaborone City Clinics through presentations and posters.

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APPENDIX

Informed Consent and questionnaire are attached.