1) TITLE: Impact of maternal HIV on *Mycobacterium tuberculosis* infection among peripartum women and their infants

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KEMRI/CDC (Kisumu)

4) FUNDING AGENCY

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5) SUMMARY

The global burden of tuberculosis disease (TB) in pregnancy\(^1\) contributes to substantial morbidity and mortality among HIV-infected women and their children.\(^2\) HIV increases progression from *Mycobacterium tuberculosis* (Mt) infection to TB,\(^3\) and the risk of TB appears higher in
pregnant/postpartum women. However, it is not known whether maternal HIV increases susceptibility to peripartum maternal or infant Mtb infection. The prevalence of Mtb infection among HIV-exposed infants is poorly defined and critical to determine due to high rates (30-50%) of progression to TB among young children. Additionally, the performance of latent tuberculosis (LTBI) diagnostic tests, including tuberculin skin tests (TST) and interferon gamma release assays (IGRA), may be affected by both HIV and pregnancy. Defining the risk of Mtb infection in these populations is important to strengthen TB prevention efforts in maternal child health (MCH) settings. Using an observational prospective parallel longitudinal cohort design, we will compare Mtb infection incidence (measured by IGRA) between HIV-infected and uninfected pregnant women and Mtb infection prevalence between HIV-exposed and unexposed children. We will also evaluate maternal HIV status and peripartum stage effect on LTBI test performance.

**Design:** Observational, prospective study of parallel longitudinal cohorts of HIV-infected and HIV-uninfected pregnant women and their infants

**Population:** HIV-infected and uninfected pregnant women and their infants

**Sample size:** 400 pregnant women (200 HIV+/200 HIV-) and 400 infants (200 HIV-exposed, 200 unexposed)

**Intervention:** Mothers will be serially tested for *M. tuberculosis* (Mtb) infection with both an interferon gamma-release assay (IGRA) and tuberculin skin test (TST) in pregnancy, 6 weeks and 12 months postpartum. Infants will be serially tested for Mtb infection at 6 weeks and 12 months of age and TST at 12 months of age.

**Study duration:** 5 years. Mothers will be followed longitudinally from enrollment in pregnancy to 1 year postpartum. Infants will be followed longitudinally from birth to 1 year of age.

**Study sites:** Kisumu County Hospital, Ahero sub-district and Bondo district Hospital, western Kenya. Additional sites if needed for enrollment goals: Siya Country Referral Hospital, Ranchuno yo sub-County Hospital, Lumumba Health Center, and Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) in western Kenya.

**Objectives:**
1) To estimate the risk of Mtb infection in HIV-infected and uninfected peripartum women
2) To estimate the risk of Mtb infection in HIV-exposed and unexposed infants
3) To determine LTBI diagnostic test performance (QFT-Plus and TST) in HIV-infected and uninfected peripartum women

**Hypotheses:**
1) HIV will be associated with increased risk of IGRA conversion by 12 months postpartum.
2) Mtb infection prevalence will be higher among HIV-exposed infants due to immunologic differences following maternal HIV exposure and/or increased exposure to active TB.
3) IGRA will identify twice as many women with Mtb infection as TST. TST/IGRA discordance will be higher in HIV-infected women, particularly during pregnancy.

**Specific Aims:**

1) Determine the effect of maternal HIV on risk and timing of maternal peripartum Mtb infection.
2) Determine the effect of maternal HIV on risk and timing of infant Mtb infection.
3) Determine the effect of HIV status and peripartum stage on LTBI test performance.
a) INTRODUCTION/BACKGROUND:
HIV-infected individuals and infants have an increased risk of progression from Mtb infection to TB disease.² Pregnant and postpartum periods have also been associated with increased risk of TB.⁴ Whether maternal HIV increases susceptibility to Mtb infection among peripartum women and their infants, and the dual roles of HIV and peripartum stage on latent TB testing are unknown. We propose to determine the impact of maternal HIV on maternal peripartum and infant Mtb infection incidence using prospective parallel longitudinal cohorts of HIV-infected and uninfected mothers and their HIV-exposed and unexposed infants. We will also evaluate the effect of maternal HIV status and peripartum stage on LTBI test performance.

b) LITERATURE REVIEW:
TB contributes significant morbidity and mortality to HIV-infected pregnant women and their children.² TB is the leading cause of mortality of HIV-infected individuals,⁸ and the third leading cause of death among women of child-bearing age in high burden areas.² Maternal HIV/TB is associated with adverse infant outcomes including prematurity, small for gestational age, vertical HIV transmission, neonatal TB, and death.²⁹ Recent estimates indicate the global TB burden in pregnancy is higher than previously thought, with >200,000 pregnant women with active TB in 2011.¹ Epide­miologic data suggest a ~2-fold increased risk of TB during late pregnancy/early postpartum.⁴ In a recent pediatric TB model, 7.5 million children were projected to have Mtb infection in 2010, of whom >650,000 developed TB.¹⁰ Pediatric TB-related mortality is underreported due to detection difficulties,¹¹ and is a top cause of respiratory death among children in sub-Saharan Africa necropsy studies.¹² Children of HIV-infected parents are at high risk of TB exposure and disease.¹³,¹⁴ Preventing TB in HIV-infected women and their children is a key step in reducing HIV-related TB morbidity and mortality.

The influence of HIV in pregnancy on Mtb infection acquisition is not well-defined and could be synergistic (Figure 1). HIV and pregnancy are both associated with progression from latent to active TB.²⁴ However, it is unclear if HIV infection or pregnancy predisposes individuals to acquiring Mtb infection.⁷,¹⁵-¹⁷ Peripartum women may be at increased risk for Mtb infection due to hormonal and immunologic changes.²⁴ Increasing levels of progesterone throughout pregnancy favor transition from Th-1 to Th-2 T-cells responses, speculated to increase risk of influenza,¹⁹,²⁰ and potentially Mtb infection.² Th-1 responses reach a nadir late in the 3rd trimester, and rebound soon after delivery.²¹ This early postpartum rebound has been described as analogous to an immune reconstitution inflammatory syndrome.¹⁸ In HIV, progression to active TB is due in part to decreases in the number and function of CD4 T-cells, impaired granuloma formation, and altered macrophage response.²² The binary concept of latent vs. active TB, especially in the context of HIV, is evolving to a continuum paradigm reflecting the complex interactions between host immune status, bacillary load, symptom development and progression to TB disease.³ It is unclear whether HIV-infected individuals are also at increased risk of acquiring Mtb infection secondary to immunocompromise; some studies suggest that a substantial proportion of incident TB cases in HIV-infected individuals are due to new TB strains (i.e. clustered infections) as opposed to
reactivation of previous infection\textsuperscript{23,24}. It is plausible that concurrent HIV and pregnancy related immunocompromise may amplify susceptibility to Mtb infection.

The effect of maternal HIV on infant Mtb risk is not well-characterized. With successful prevention of maternal to child transmission (PMTCT) programs, the population of HIV-exposed but uninfected (HEU) children is growing. These children have increased risk of mortality compared to HIV-unexposed children,\textsuperscript{25} and altered immune responses to immunizations and infections, with conflicting data regarding decreased cytokine response to Bacille Calmette-Guerin (BCG) vaccine.\textsuperscript{26,27} It is unclear if altered BCG response is linked with Mtb infection risk or progression. HEU children may be at increased risk of Mtb infection due to altered immunity and TB exposure associated with maternal HIV.

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Population</th>
<th>Conversion rate</th>
<th>Conversion cofactors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aichelburg 2014\textsuperscript{15}</td>
<td>Vienna, Austria</td>
<td>HIV+ adults attending outpatient HIV clinic in low TB incidence setting</td>
<td>9% over 24 months</td>
<td>High TB incidence birth country, injection drug use</td>
</tr>
<tr>
<td>Pullar 2014\textsuperscript{28}</td>
<td>Norway</td>
<td>HIV+ adults attending outpatient HIV clinic in low TB incidence setting</td>
<td>7% over 12 months</td>
<td>Unknown</td>
</tr>
<tr>
<td>Jonnalagadda, LaCourse 2015\textsuperscript{29}</td>
<td>Nairobi, Kenya</td>
<td>HIV+ pregnant women tested at 32 weeks gestation and 1 year postpartum</td>
<td>17% over ~14 months</td>
<td>HIV+ partner, flush toilet, maternal illness and cough during follow-up</td>
</tr>
<tr>
<td>Mathad 2014\textsuperscript{7}</td>
<td>Pune, India</td>
<td>HIV+ pregnant women tested at pregnancy, delivery and postpartum, nested longitudinal cohort</td>
<td>38% between pregnancy and postpartum (unpub)</td>
<td>Not identified for longitudinal cohort. Cross-sectional cohorts: postpartum stage, urban/periurban residence</td>
</tr>
<tr>
<td>LaCourse 2016 (unpub)</td>
<td>Western Kenya</td>
<td>HIV+ pregnant women tested in pregnancy and 6 weeks postpartum</td>
<td>4% between pregnancy and 6 weeks postpartum 13.4/100 person-years</td>
<td>Not identified for conversion due to limited power.</td>
</tr>
</tbody>
</table>

Serial detection of TB-immune responses using IGRA can contribute to estimation of Mtb infection. Historically, diagnosis of Mtb infection was based on TST, but this method can produce false positive results with cross-reactivity to BCG and non-tuberculosis mycobacteria (NTM), and false negative results due to impaired cellular immunity.\textsuperscript{30} IGRA measures \textit{in vitro} release of interferon-gamma (IFN-γ, a primarily Th-1 response) after stimulation by Mtb-specific antigens, therefore is not cross-reactive with BCG or most non-tuberculosis mycobacteria, and may be less influenced by anergy.\textsuperscript{30,31} Multiple studies in HIV-infected adults have assessed TST before and after initiation of ART.\textsuperscript{28,32,33} In this context, TST is unable to discriminate new Mtb infection from immune reconstitution of TST response following ART. Combined IGRA and TST testing can provide information regarding incremental value of a new test using latent class models that yield more realistic estimates of Mtb infection by treating both the index test (IGRA) and reference test (TST) as imperfect tests.\textsuperscript{34,35} Although a high proportion of IGRA reversions (positive to negative) have been reported in healthcare workers\textsuperscript{36,37} and HIV-infected individuals in low TB burden settings,\textsuperscript{15,28,38} reversion appears less frequent in high TB burden settings, particularly with baseline positive IGRA well above the cut-off threshold.\textsuperscript{39-41} There are few estimates of Mtb infection using serial IGRA among HIV-infected and/or peripartum individuals in high burden settings (Table 1). QuantiFERON-TB Gold (QFT) IGRA, measures the amount of INF-γ released by primarily CD4+ T helper lymphocytes after TB-specific antigens (ESAT-6, CFP-10 and TB7.7)
stimulation. QuantiFERON-TB Gold Plus (QFT-Plus) measures INF-γ released by CD8+ cytotoxic T lymphocytes as well, after stimulation with the same antigens, which may have increased sensitivity in populations with lower CD4 counts including HIV. QFT IGRA correlated better with TB contact compared to TST among HIV-infected and uninfected children in Cape Town, South Africa. Longitudinal IGRA evaluation provides an opportunity to estimate incident Mtb among HIV-infected peripartum women and their HIV-exposed children.

Data are limited on Mtb infection incidence among HIV-infected peripartum women and their children. Among HIV-infected women in Kenya with negative IGRA during pregnancy, 17.4% had an IGRA conversion at 12 months postpartum (Table 1). Notably, IGRA conversion was substantially above the positive threshold. Among those with positive IGRA, the majority remained positive throughout the postpartum period, suggesting stability in positive postpartum IGRA responses. In a study of 6-month old HIV-exposed Kenyan infants, 10.9% were IGRA positive, suggesting a Mtb cumulative infection rate of >20% at 1 year. In these peripartum and pediatric cohorts, most IGRA conversions occurred without an identified TB contact. In South Africa and Botswana, among 3-4 month old HEU children screened for TB exposure before isoniazid prophylaxis trial entry, >10% had a TB contact. In this trial, even after negative study entry screens for TB exposure, 7.3% of HEU infants developed TB disease and 2.6% were diagnosed with Mtb infection by TST. In a contemporary cohort of pregnant HIV-infected women in Kenya, 3.8% converted from both negative TST/IGRA to IGRA+ by 6 weeks postpartum, for an estimated incidence of detected LTBI infection of 13.4/100 person-years (LaCourse, unpub). These data indicate Mtb infection risk is high among HIV-infected peripartum women and their children, despite lack of obvious contact.

Detecting LTBI, particularly recent infection, can identify those individuals who may most benefit from preventive therapy. Although a recent trial of ART and isoniazid preventive therapy (IPT) vs. ART alone for the prevention of TB showed benefit irrespective of TST response, most studies demonstrate greater IPT efficacy among those with positive TST. A 2010 systematic review of RCTs reported 62% reduction in active TB among HIV-infected TST positive adults not on ART. This TB risk was further reduced to 72% with longer duration IPT (36 months) in Botswana. The WHO recommends HIV-infected adults without evidence of active TB receive IPT, and that benefit is greatest for those with positive TST. Immediate ART plus IPT resulted in fewer TB cases vs. deferred ART + IPT or immediate ART without IPT in the Ivory Coast, with greater benefit among those who were IGRA positive. Following IGRA conversion, South African adolescents had an 8-fold higher risk of developing active TB within 2 years compared to non-converters. Children <1 year have a 30-50% risk of progression from Mtb infection to disease. Identification of early Mtb infection is important, due to the substantially increased risk of developing TB within the first 1-2 years following infection.

TB cases in HIV-infected individuals may be due to new infection and TB risk remains high despite ART. Molecular fingerprinting studies indicate TB cases in HIV-infected individuals in sub-Saharan Africa are more likely due to new infection as opposed to reactivation. Although ART decreases risk of active TB by 67% (with TB risk declining with increase in CD4 and ART duration), the risk of active TB remains significantly elevated despite CD4 recovery on ART and even among those initiating ART at CD4>350, compared to HIV-uninfected individuals. Susceptibility to TB may be related to qualitative T-cell dysfunction and may partially explain why HIV-infected individuals on ART remain at risk of TB even after CD4 count recovery. These studies, coupled with universal ART coverage modeling data, indicate that ART alone will be insufficient to prevent future HIV-associated TB. Given the high proportion of TB due to recent transmission among HIV-infected individuals, including those on ART, early
identification of recent Mtbi infection is an important component of TB prevention and control strategies in PMTCT programs.

The effect of both HIV and peripartum stage on LTBI diagnostic test performance is unknown. In a meta-analysis of IGRA in HIV-infected individuals, pooled sensitivity of IGRA Quantiferon Gold (QFT) in low-income countries was 60% (95% CI 47–75%). In more recent studies, agreement between TST and QFT in HIV-infected individuals was 55-73% (kappa 0.29-0.51). In pregnancy, cellular expression of IFN-γ is reduced in the later stages of pregnancy with postpartum rebound. A significant decrease in in-vitro lymphocyte response to tuberculin purified protein derivative was seen in previously TST positive women in the US during late pregnancy and delivery, which returned to early pregnancy levels 24 hours postpartum. Among HIV-negative women in India, the proportion of positive IGRA was significantly higher throughout the peripartum period with increased IGRA positivity and IGRA+/TST- discordance postpartum (Figure 2). In a cross-sectional study in India, there was discordance between TST and IGRA regardless of HIV status, with higher discordance among HIV-infected women (kappa 0.22 vs. 0.40) indicating a reliance on TST for LTBI screening would result in >50% fewer HIV-infected pregnant women being treated with IPT (29% IGRA+ vs. 11% TST+, p=0.01) (Mathad & Gupta, CROI, 2014). These studies longitudinally evaluated HIV-negative peripartum women and cross-sectionally compared HIV-infected and uninfected pregnant women in India.

In our recently completed pilot study of HIV-infected peripartum women in western Kenya, more women were QFT+ than TST+ in pregnancy (n=96, 35.4% vs. 13.5%, p=0.001) and 6 weeks postpartum (n=88 29.6% vs. 14.8%, p=0.001) (LaCourse, unpub). Among 18 consistently QFT+ women, 8 (44%) converted from TST- to TST+, with improved test agreement postpartum (56.9%, \(\kappa=0.20\) to 82.4%, \(\kappa=0.60\), 95% CI 0.42-0.77). Intriguingly, mean QFT mitogen (4.46 vs. 7.64 IU/mL, p<0.001) and Mtbi-Ag (1.03 vs. 1.54 IU/mL, p=0.03) responses were lower among all women retested in pregnancy vs. postpartum, (mitogen: 4.46 vs. 7.64 IU/mL, p<0.001, Mtbi-Ag: 1.03 vs. 1.54 IU/mL, p=0.03), and specifically among persistently QFT+ women (Mtbi-Ag: 3.46 vs 4.48 IU/mL, p=0.007). QFT indeterminate rate was higher in pregnancy (16%) compared to postpartum (0%) due to lower mitogen response. These lower QFT Mtbi-Ag and mitogen responses in pregnancy compared to postpartum suggest pregnancy-associated immunologic changes may influence LTBI test performance in HIV-infected women. Larger cohorts with longer duration of follow-up are needed to probe correlates of test discordance and conversion as well as to investigate whether maternal HIV status impacts LTBI diagnostics. There are no published studies longitudinally comparing TST and IGRA in women who are HIV-infected and pregnant; the combined effects of pregnancy and HIV on LTBI tests are not known.
HIV and pregnancy are associated with increased risk of progression to TB disease, but the risk of peripartum maternal and infant Mtb infection in the setting of maternal HIV is unknown. Identifying Mtb infection is important for potential interventions to decrease progression to TB disease in these high-risk groups.

Our preliminary data indicate IGRA conversion is high among peripartum HIV-infected Kenyan women (17% between pregnancy and 12 months postpartum), and Mtb infection prevalence is substantial in their children (10% at 6 months of age). Positive IGRA during pregnancy was associated with a 3 to 5-fold increase in postpartum maternal and infant TB and mortality. In our recently completed pilot study of pregnant HIV-infected women, TST missed >60% of Mtb infection detected by IGRA (LaCourse, IAS 2015). However, lower mean QFT Mtb-Ag and mitogen levels and higher rates of indeterminates (due to low mitogen levels) in pregnancy compared to postpartum, suggest that interferon-gamma release assays are also likely impacted by pregnancy-related immunologic changes. These data demonstrate significant risk of Mtb infection and TB disease in HIV-infected mothers and their infants.
8) HYPOTHESIS & STUDY QUESTIONS:
Hypothesis 1: HIV will be associated with increased risk of IGRA conversion by 12 months postpartum.
Hypothesis 2: Mtb infection prevalence will be higher among HIV-exposed infants due to immunologic differences following maternal HIV exposure and/or increased exposure to active TB.
Hypothesis 3: IGRA will identify twice as many women with Mtb infection as TST. TST/IGRA discordance will be higher in HIV-infected women, particularly during pregnancy.

9) OBJECTIVES
a) BROAD OBJECTIVES:
The broad objective of this study is to determine the impact of maternal HIV on maternal peripartum and infant Mtb infection incidence and to evaluate the effect of maternal HIV status and peripartum stage on LTBI test performance. Defining the risk of Mtb infection in these populations is important to strengthen TB prevention efforts in maternal child health (MCH) settings.

b) SPECIFIC OBJECTIVES:
1) To estimate the risk of Mtb infection in HIV-infected and uninfected peripartum women
2) To estimate the risk of Mtb infection in HIV-exposed and unexposed infants peripartum women
3) To determine the LTBI diagnostic test performance (QFT-Plus and TST) in HIV-infected and uninfected peripartum women

10) STUDY DESIGN AND METHODOLOGY:

Table 2: Overall Study Strategy

| Study Design: | Observational, prospective parallel longitudinal cohorts |
| Primary Outcomes: | AIM 1: Maternal peripartum Mtb incidence (IGRA)  
AIM 2: Infant Mtb prevalence (IGRA)  
AIM 3: Maternal peripartum IGRA/TST discordance |
| Population: | HIV-infected and uninfected pregnant women and their infants |
| Exclusions: | Women with active TB in past 1 year or on enrollment |
| Target enrollment: | Aim 1: ~330 pregnant women (~165 HIV+/165 HIV-)  
Aim 2: 400 infants (200 HIV-exposed, 200 unexposed)  
Aim 3: 400 pregnant women (200 HIV+/200 HIV-) irrespective of enrollment IGRA/TST status |
| Follow-up duration: | Mothers: ~15 months (pregnancyb - 1 year postpartum)  
Infants: 12 months (birth - 1 year) |
| Sampling framework: | Consecutive enrollment of pregnant women in antenatal care and their infants, Kisumu County Hospital, Ahero Sub-district and Bondo District Hospitals, Nyanza region of western Kenya. Additional sites if needed for enrollment goals: Siya Country Referral Hospital, Ranchuonyo sub-County Hospital, Lumumba Health Center, and Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) |

AIMS 1 & 2: Longitudinal assessment of Mtb infection risk among HIV-infected and HIV-uninfected peripartum women and their children. **Aim**
1: Maternal cohorts (Mtb incidence): To estimate maternal Mtb incidence, we will identify women with negative IGRA at enrollment (~330 women), and serially test them at 6 weeks and 1 year postpartum with IGRA. Aim 2: Infant cohorts (Mtb prevalence): We will serially test infants born to all enrolled mothers (200 HIV-exposed, 200 HIV-unexposed) at 6 weeks and 12 months with IGRA.

Aim 3: Prospective study of IGRA/TST concordance among peripartum HIV-infected and HIV-uninfected women. We will measure TST/IGRA concordance by peripartum stage in 200 HIV-infected and 200 HIV-uninfected pregnant women in pregnancy, 6 weeks and 1 year postpartum.

b) STUDY AREA DESCRIPTION

This study will be conducted in Nyanza Province at 3 public sector hospitals: Kisumu District Hospital, Bondo District Hospital, and Ahero sub-District Hospital. We have enrolled women and infants in longitudinal studies at these sites for >4 years. We also enroll at Siya Country Referral Hospital, Ranchuonyo sub-County Hospital, Lumumba Health Center, and Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) if needed to meet enrollment goals. The area is suitable for this research study because of the high HIV-1 prevalence in the region (19-26% antenatal prevalence), high clinic turnover (approximately 200 new pregnant women per month) and the relevance of the research to the local population. The three hospitals are close in proximity to the KEMRI/CDC laboratory, which will process and store specimens collected during the study.

Kenya is a high TB burden country (annual TB incidence of 268/100,000). Nyanza has a high antenatal HIV prevalence (19-26%), and TB-HIV co-infection. The proportion of TB cases with HIV is ~68%. In our prior study, the burden of culture-confirmed pulmonary TB was ~2.4% among HIV pregnant women at sites in this area. The cumulative Mtb infection of HIV-exposed infants at 6 months is >10%. A large study from AMPATH (Western Kenya) estimated TB disease annual incidence among HIV-infected children (median age 1 year) to be 17%.

c) STUDY POPULATION:

The study population will comprise HIV-infected and HIV-uninfected pregnant women seeking antenatal care services at three public hospitals in Nyanza Province and their children. The population attending these clinics is reflective of women living in Nyanza Province; the source population is rural, diverse in ethnicity, and of generally low socioeconomic status.

Inclusion Criteria:
- Pregnant women >16 years of age (between 20-34 weeks gestation) and their infants will be eligible for enrollment.
- Pregnant and plans to remain in the area with their infants until at least 12 months postpartum
- Willing to have serial visits at MCH clinic with serial Mtb infection testing and TB symptom screening until 12 months postpartum

Exclusion Criteria:
- Not pregnant
- Women with TB in the past year or found to have TB on enrollment
- Resides outside the clinic catchment area
- Intends to move from the clinic catchment area during pregnancy and 12 months postpartum period
• Is unable or unwilling to participate in serial visits to the MCH clinic during pregnancy and the 12 month postpartum period

d) SAMPLE SIZE DETERMINATION AND FORMULAS USED:


Aims 1 & 2 Sample size and power calculations:

Aim 1: We expect ~165 women will have initial negative IGRA in each cohort of 200 women. We will have 80% power to detect a ~6.0-9.6% increase in proportion of IGRA positivity at ~15 months of follow-up in HIV-infected peripartum women if IGRA conversion is 1-5% among HIV-uninfected mothers. (Table 3). Expected outcomes: Aim 1: We have >80% power to detect a ~6% increase in Mtb infection HIV-infected women compared to HIV-uninfected women (7% vs. 1% at 1 year postpartum), which is likely adequate given our pilot study which found 17% IGRA conversion at 1 year postpartum in HIV-infected peripartum women.

<table>
<thead>
<tr>
<th>Proportion of IGRA conversion in HIV-uninfected</th>
<th>No. per maternal cohort*</th>
<th>Min detectable difference in proportion of IGRA conversion in HIV-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>165</td>
<td>6.0%</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>6.5%</td>
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</table>

* excludes women IGRA+ at enrollment, and potential lost to follow-up

Aim 2: With 200 children in each infant cohort we will have 80% power to detect ~5.2 to 9.0% increase in prevalence of IGRA positivity at 12 months of follow-up in HIV-exposed infants, if IGRA conversion is 1-5% in infants of HIV-uninfected mothers (Table 4). Expected outcomes: Aim 2: We have >80% power to detect a ~5% increase in Mtb infection between HIV-exposed and HIV-unexposed infants (6.2% vs. 1%), which is likely adequate given our pilot data demonstrating 20% IGRA positive among HIV-exposed children at 1 year of age. If Mtb infection incidence difference is smaller, we will still gain novel information that can be correlated to maternal/infant cofactors.

Aim 3: Prospective study of IGRA/TST concordance among peripartum HIV-infected and HIV-uninfected women.

Aim 3 Sample size and power calculations:

Aim 3: With 200 women in each cohort, and assuming the true κ value is 0.50, a two-sided 95% confidence interval for the κ statistic would be +/-0.16 for an estimated LTBI prevalence of 20%. Precision will improve with higher LTBI prevalence and higher or lower kappa. Expected outcomes: Aim 3: We expect that IGRA will identify 2-fold as many women with Mtb infection compared to TST. Our pilot data indicates that TST identifies <60% of HIV-infected women with positive IGRA. We expect κ will be low (0.20-0.40), and discordance will be higher among HIV-infected women.
infected compared to HIV-uninfected women, and higher during earlier compared later postpartum periods.

e) SAMPLING METHOD:

Since the study aims to measure incidence and prevalence of Mtb infection among peripartum women and their infants, we aim to obtain a sample of subjects who are representative of the general population. We will consecutively enroll eligible pregnant women and their subsequent infants from maternal health and prevention of maternal to child health clinics. Recruiting from maternal-health clinics will help ensure representativeness of the study population, because the existing public sector infrastructure serves the majority of women in the population who become pregnant and includes in-built frequent follow-up to 12 months postpartum for delivery of childhood immunizations and infant growth monitoring.

f) DEFINITION OF CASES/CONTROLS IF APPLICABLE:

Not applicable.

g) RECRUITMENT AND CONSENTING PROCEDURES:

Recruitment: HIV-infected and uninfected pregnant women presenting for antenatal care at Kisumu District Hospital, Bondo District Hospital, and Ahero sub-District Hospital will be informed about the study and offered participation by antenatal clinic staff/nurses and referred to the study staff for more information about the research. We also recruit at Siya Country Referral Hospital, Ranchuonyo sub-County Hospital, Lumumba Health Center, and Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) if needed to meet enrollment goals. Patients who are interested in learning more about the study will be able to complete the informed consent process and the study enrollment visit on the same day. Potential subjects recruited for this trial are limited to those persons who are pregnant, ages 16 or older, receiving antenatal services, and their children who are born at the end of the pregnancy during which the subject is enrolled. Potential subjects will have access to all available antenatal, postnatal, pediatric, HIV, and TB services regardless of their decision to participate in the research. Access to antenatal services or maternal child health services will not be delayed based on inclusion in the study.

Following informed consent and study screening procedures, eligible pregnant women will be offered enrollment into the study. Informed consent will occur in a one-on-one counseling session with a study staff person in either Kiswahili, Dholuo or English according to participant’s preference. During the informed consent counseling, the counselor will describe the study in detail, review the consent form, including reviewing expected benefits and possible risks of participation, and answer all questions a subject may have. For women who are not literate, the consent will be read to them in the appropriate language of their choosing with a witness independent of the study present. For women who cannot write, an inked thumbprint will be used in lieu of signature. Women will provide consent for their infants. Subjects will be notified that they can withdraw consent for participation in the study at any time. A copy of the consent form in the language of preference will be offered to the participant. Once patients consent to study participation, they will be asked to sign an informed consent form. Informed consent will occur in a one-on-one counseling session with a study staff person and will occur in either Kiswahili, Dholuo or English according to participant’s preference.

h) DATA COLLECTION PROCEDURES (clinical and non-clinical, field, data collection instruments):

Follow-up schedule: For the proposed study, we will incorporate Study visits to coincide with scheduled MCH visits. Mothers will have study visits at enrollment, 6 weeks, 6 months and 12
months postpartum. TST and IGRA will be performed on enrollment, 6 weeks and 12 months postpartum. Infants will have study visits at 6 weeks (enrollment), 6 months, and 12 months with IGRA performed at 6 weeks and 12 months and TST performed at 12 months. Please see Figure 3 for Study Flow.
Clinical Procedures:

**Maternal cohorts:** Pregnant women attending ANC care will be recruited, and enrolled if eligible, after written informed consent. At enrollment, household locator information, HIV care medical identification number, and cell-phone contacts will be obtained to facilitate tracing. A study nurse will administer a standardized questionnaire that addresses sociodemographic, clinical, obstetric and HIV-related factors, TB exposure and history, and maternal and household member TB symptoms (using WHO symptom screen) (Table 5). Mothers with suspected TB on enrollment will be referred to the TB program and if found to have active TB, will be ineligible for participation. At each visit mothers will undergo physical examination with BMI calculation; medical records will be used to abstract data on ART regimen, other medications, maternal HIV viral load and CD4 cell counts by trained study staff. HIV-infected women found to have positive TST or IGRA testing will be referred for IPT. Please see Table 6: Study visits and planned procedures.

**Infant cohorts:** Infants will be enrolled at the 6 weeks immunization visit where infant medical records and MCH cards will be used to abstract maternal PMTCT ART, infant PMTCT prophylaxis, infant HIV status, birth weight, BCG vaccination date, intercurrent illnesses, and vaccines (Table 5). At each visit infant examination will include growth measures, questionnaire addressing infant feeding (including breastfeeding status), and symptom clinical review (including cough, fever, weight loss). Detailed TB exposure information will be recorded for both mothers and infants including relationship, duration of contact to TB suspect, as well as reported TB symptoms among household members. Please see Table 6: Study visits and planned procedures.

Study participants will be reimbursed approximately 300 Kenyan Shillings (KSH) for transport and effort at each study visit. Payment will be administered at the conclusion of the study visit.
Table 5: Data Collection

**Source: Locator survey**
- Detailed information for locating subjects including address, directions and mobile phone number.

**Source: Standardized Questionnaire**
- **Anthropometric:** Weight, height, MUAC
- **Sociodemographic:** maternal age, education level, rooms in household, number of household members, household water/electricity source
- **Pregnancy:** gestational age, pregnancy history including history of complication.
- **TB:** TB symptom screen, TB infection or disease and treatment history, family history of TB disease, contact TB history including history of TB disease or symptoms of TB disease in household members and known contact with TB patients over last 2 years
- **HIV:** HIV status, treatment history, history of opportunistic infections, partner/spouse’s HIV status (if known), isoniazid preventive therapy
- **General health:** medication and other general medical conditions, breastfeeding and infant feeding

**Source: MCH/PMTCT/HIV/TB medical records**
- **Anthropometric:** Weight, height
- **Pregnancy:** gestational age, pregnancy complications, routine pregnancy labs, medications
- **TB:** TB infection or disease and treatment history, TB w/u lab results, TB treatment outcome
- **HIV:** CD4 count, viral load, date of HIV diagnosis, ART history, current medications, co-trimoxazole prophylaxis, isoniazid preventive therapy, h/o opportunistic infections
- **General health:** medication and other general medical conditions, breastfeeding and infant feeding, immunizations
## Table 6: Study visits and planned procedures

| Maternal cohorts | Antenatal | Postpartum |  |  |
|------------------|-----------|------------|  |  |
|                   | VISIT 1   | 48-96 hrs | VISIT 2 | 48-96 hrs | VISIT 3 | 6 month postpartum | VISIT 4 | 12 month postpartum | 48-96 hrs |
|                   | Pregnancy | after | 6 weeks postpartum | after | 6 month postpartum | after |
| Enrollment        | x         |       |           |           |           |       |
| Sociodemographic survey | x         | x | x |
| Health history    | x         | x | x |
| Physical exam     | x         | x | x | x |
| TB exposure screen | x         | x | x | x |
| TB symptom screen | x         | x | x | x |
| Blood draw (10 ml) | x         | x |       |       |       |       |
| Breast milk       |           |       |           |           |           |       |
| TST placed        | x         | x |       |       |       |       |
| TST read          |           | x | x |       |       | x |
| Referral for HIV testing* |           | x | x | x | x |
| Infant cohorts    |           | 6 weeks | 6 months | 12 months | 48-96 hrs |
| Enrollment        |           | x       |           |           |           |       |
| Sociodemographic survey |           | x | x | x | x |
| Health history    |           | x | x | x |
| Physical exam     |           | x | x | x |
| TB exposure screen |           | x | x | x |
| TB symptom screen |           | x | x | x |
| Blood draw (5 ml) |           | x |       |       |       |       |
| TST placed        |           |       |           |           |           |       |
| TST read          |           |       |           |           |           | x |
| Referral for HIV testing** |           | x | x | x | x |

*For women who are HIV-uninfected on enrollment
** For children who are HIV-exposed
Laboratory/testing procedures:

**Maternal cohorts:** Mothers will have study visits at enrollment, 6 weeks, 6 months and 12 months postpartum. TST and IGRA will be performed on enrollment, 6 weeks and 12 months postpartum (Figure 3, Table 6). Breast milk will be collected at 6 weeks postpartum. HIV-negative women will be referred to MOH clinics for HIV testing at 6 weeks, 6 and 12 months postpartum.

**Infant cohorts:** Infants will have study visits at 6 weeks (enrollment), 6 months, and 12 months with IGRA performed at 6 weeks and 12 months (Figure 3, Table 6). Due to the high rates of BCG cross-reactivity after recent immunization, we will not perform TST in infants until 12 months of age. HIV-exposed children will be referred for HIV PCR testing at 6 weeks, and HIV testing at 1 year per national guidelines. In the case of changing HIV-testing guidelines we will refer mothers and infants according to the most up-to-date guidelines at the facilities where they are enrolled.

**Blood:** Maternal (10 ml) and infant (5 ml) blood will be collected for IGRA assays and flow cytometry. For IGRA QFT-Plus assays, blood is collected in collection tubes (nil, mitogen, TB antigen 1 [ESAT-6 and CFP-10 CD4 peptides], TB antigen 2 [ESAT-6, CFP-10 CD4 and CD8 peptides]) and processed within 16 hours of collection per manufacturer recommendations. A response of ≥0.35 IU/ml to TB antigens in either TB 1 or TB 2 (with nil <8 IU/ml and positive mitogen control) will be considered positive. Blood for IGRA will also be drawn in the event of a concern for active TB diagnosis. For flow cytometry blood is processed to separate into PBMC and plasma.

**Breast milk collection.** Up to 30 mls of breast milk will be collected from mothers at 6 weeks postpartum and evaluated for maternal antibodies to TB.

**TST:** TST will be placed and read by a study nurse within 48-96 hours. Trained study personnel will inject 0.1 ml (5 international units) of the designated PPD intradermally to the volar surface of the designated forearm using the Mantoux method. The amount of swelling at the site (induration) will be interpreted at 48-96 hours following TST placement by study staff. TST of >5 mm will be considered positive in HIV-infected women, and >10 mm among women and infants without HIV. TST conversion will be defined as >5 mm of induration regardless of previous results in HIV-infected and an increase of >10 mm in HIV-uninfected women.

TST is contraindicated only for persons who have had a severe reaction (e.g., necrosis, blistering, or ulcerations) to a previous TST; this is a rare occurrence (less than 1%). Prior to TST placement, subjects will be asked if they have had a severe reaction in the past. Subjects with a history of severe reaction will not have a TST placed but will remain in the study. Subjects who experience a dermatologic adverse effect from the TST will be treated in the study clinic for care and management.

All Specimens will be handled using universal precautions, treated as potential Group 3 pathogens in lab, and will be handled by staff trained in Good Laboratory Practices.

**i) VARIABLES:** dependent, independent, confounders

Several research questions will be addressed in this study. The main study variables and their associated aims are detailed in Table 7.

**Table 7. Study variables used to address the specific aims.**
Aim | Outcome (Dependent variable) | Exposure (Independent variable) | Measurement | Potential Co-factors
--- | --- | --- | --- | ---
Aim 1 | Maternal Mtb infection incidence | Maternal HIV status | IGRA | Maternal age, CD4, viral load, ART use, partner HIV status, TB exposure, household TB symptoms, IPT, peripartum stage, household crowding, employment, magnitude of IFN-γ response
Aim 2 | Infant Mtb infection prevalence | Infant HIV exposure status | IGRA | Maternal Mtb infection status, prevalent vs. incident maternal Mtb infection, maternal/infant IPT, CD4, and ART; household crowding, TB exposure, paternal HIV status, infant HIV status, magnitude of IFN-γ response
Aim 3 | Maternal LTBI diagnostic performance | Maternal HIV status | IGRA | TST | Maternal age, CD4, ART use (among HIV-infected), peripartum stage, time since TB exposure, magnitude of IFN-γ response, prevalent vs. incident maternal Mtb infection

**j) MATERIALS:**

**Equipment:** No funds are requested for equipment. Funds will be used to purchase necessary supplies. Lab testing will occur at the internationally accredited KEMRI/CDC lab with available equipment.

**Personnel:** The grant award includes support for UW investigators, clinic personnel, the data team, and a study coordinator. Study personnel working in Kenya will be hired through UON/KNH or UW-Kenya according to standard procedures.

**k) TRAINING PROCEDURES:**

Drs. Sylvia LaCourse, John Kinuthia and Daniel Matemo will supervise training of clinical personnel in study procedures. This will include research ethics, LTBI/TB counseling and testing, specimen collection, and completion of study questionnaires and report forms. We will offer PMTCT and ANC staff one hour of training on active TB, LTBI and the impact of TB during pregnancy and in early childhood.

We will ensure that study personnel are trained in communicating important study results to MCH providers. Significant results include: the presence of symptoms concerning for active TB according to Kenya National Guidelines, the results of sputum smear evaluation (AFB-smear and –culture), and the results of tuberculin skin testing. For subjects diagnosed with active TB, we will facilitate referrals to the National Treatment Programme for prompt initiation of treatment. As we anticipate that our study may increase referrals for LTBI prophylaxis and treatment of active TB, we will discuss our study with the staff at the hospital’s TB and HIV comprehensive care clinics prior to study enrollment.

**l) QUALITY ASSURANCE PROCEDURES:**

**Clinical care:** The study will adhere to Government of Kenya guidelines for the care of pregnant/postpartum women and their infants. Data collected as part of the study will be abstracted from the mother’s “Mother & Child Health Booklet” as well as the MCH clinic’s medical records. Counseling and testing for HIV will be performed in accordance with government-approved MCH guidelines. HIV-infected study participants will receive their HIV medicines at their...
local hospital’s Comprehensive Care Clinic/MCH clinic, enabling reporting of antiretroviral therapy and follow-up in accordance with the national AIDS strategy.

Procedures to minimize risk of HIV, vertical transmission of HIV
HIV-uninfected women will be counseled in ways to reduce their risk of HIV acquisition, including condom use. HIV-infected women will be counseled on the prevention of mother to child transmission per Kenyan guidelines. Previously HIV-negative women and HEU infants found to be HIV-infected during the study will be immediately referred to appropriate HIV services. HIV evaluation and treatment is free per Kenyan National HIV guidelines.

Procedures to minimize risk of progression from latent to active TB
HIV-infected women with evidence of latent TB, or development of new Mtb infection (by either positive TST or IGRA) during the study will be referred to the HIV comprehensive care clinic on site to receive isoniazid preventive therapy per Kenyan guidelines. Children identified as having close contact with a person with TB will also be referred to the TB program clinic for isoniazid preventive therapy per Kenyan guidelines. All subjects identified as having potential active TB will be referred to the TB program for further investigation as well as treatment as required per Kenyan National TB guidelines including CXR and Xpert as necessary. Isoniazid prevention therapy and TB evaluation and treatment is free per Kenyan National TB guidelines.

Procedures to minimize risk of pregnancy complications
Women with high-risk pregnancies, or risks of complications will be referred to deliver at their closest MCH-linked maternity center (Kisumu County Hospital, Bondo District Hospital or Ahero sub-District Hospital, Siya Country Referral Hospital, Ranchuonyo sub-County Hospital, Lumumba Health Center, and Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH))). Women or children developing other unexpected health problems that are not addressable within the study clinic will be referred to the appropriate clinic within the hospital.

Procedures to ensure newly diagnosed HIV-infected women receive HIV care
Enrolled HIV-uninfected pregnant women will be referred for HIV tests through MCH trained HIV counselors at enrollment, 6 weeks, 6 months and 12 months postpartum. If any women are found to be HIV-infected they will be referred for HIV care (including prevention of mother to child transmission of HIV services) through the HIV care clinic.

Procedures to ensure newly diagnosed HIV-infected children receive HIV care
Enrolled HIV-unexposed infants referred for HIV tests through MCH trained HIV counselors at 6 weeks and 12 months of age. If any of these children are found to be HIV-infected they will be referred immediately for HIV care through the Pediatric HIV care clinic.

Adherence to protocol: Weekly reporting of enrolment, follow-up, medical complications, laboratory results, and specimen collection will enable us to monitor that the study is running according to approved protocols. Frequent reporting will also enable us to quickly respond to any problems that arise during the study.

Laboratory quality control: The KEMRI/CDC laboratory that will process the specimens and conduct most of the laboratory tests is ISO certified, and participates in external quality control verification.

11) ETHICAL CONSIDERATIONS
a) Consent explanation
**Study Protocol**

- **Title:** Impact of maternal HIV on Mycobacterium tuberculosis infection among peripartum women and their infants

- **Introduction:** "We are asking you and your child to be in a research study. The purpose of this form is to give you the information you will need to help you decide whether you and your child will be in the study or not. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions, you can decide if you want to be in the study or not. This process is called “informed consent.” This form serves as both as a record of your consent to be in the study and as a parental permission form. We will give you a copy of this form for your records."

**The word “you” in this form refers to you and your child.**

**What you should know about this study:**

- This form explains what would happen if you join this research study.
- Please read it carefully. Take as much time as you need.
- Please ask the research team questions about anything that is not clear.
- You can ask questions about the study at any time.
- If you choose not to be in this study, it will not affect any other care received at clinic.
- If you say ‘Yes’ now, you can still change your mind later.
- You can quit the study at any time.
- You would not lose benefits or be penalized if you decide not to take part in the study or to quit the study later.

- **Objectives of the study:** “The goal of any research study is to answer questions. We (the research team listed on the front of this form and our staff) are doing this research study to answer the following question:

**Objectives of the study:** Does having HIV increase the risk of getting Mycobacterium tuberculosis (MTB) infection in peripartum women and their infants?

**Infection with the organism called MTB Mycobacterium tuberculosis (MTB) causes the disease called tuberculosis (TB).** Once infected with MTB, some people go on to get TB disease but not all. Young children, people with HIV, and women who are pregnant more likely to get TB disease because their body defenses are sometimes weak. Pregnancy may also increase the risk of developing TB. However, we do not know if HIV increases the risk of getting MTB infection in pregnancy or in general.”

- **Benefits:**

  **Potential Benefits for You:**
  Being in this study might benefit you in the following ways:
  - Close monitoring of TB symptoms which will prompt referral to appropriate screening and treatment facilities
  - Referral for serial testing of HIV (if currently negative) which may identify newly infected women and their infants.
  - HIV-infected women with positive TSTs as well as infants found to have recent TB contacts, may benefit from referral for isoniazid prophylaxis, as well as from focused questioning regarding TB risk factors and prompt referral to the TB program if concern for active TB arises.

  **Potential Benefits for Others:**
• The research will provide important information regarding whether HIV in pregnancy increases susceptibility to MTB infection in peripartum women and their infants, as well as if HIV affects how well tests for TB infection work.
• This information could benefit other women and their children in areas of high HIV/TB burden.”

-Risks:
“What are the potential harms or risks if I join this study?
There are potential harms or risks if you take part in this study. Some are common and some are rare.

Potential Harms and Discomforts (from the most common, to the most rare):
• Local irritation due to blood draw.
• Local irritation due to TST
• Loss of confidentiality

We do not anticipate any study-related physical adverse effects to participants. We believe the risk of loss of confidentiality is low due to measure we have in place to protect your privacy.”

-Compensation mechanism: “Study participants will be reimbursed approximately 300 Kenyan Shillings (KSH) for transport and effort at each study visit. Payment will be administered at the conclusion of the study visit.”

-Alternative treatments: “Whether or not you decide to participate in this research study, you can continue to receive your regular mother-child health care at this clinic.”

-Voluntarism:
“Whether or not you decide to participate in this research study, you can continue to receive your regular mother-child health care at this clinic.”

“When we have answered all your questions, you can decide if you want to be in the study or not. This process is called “informed consent.”

• “If you choose not to be in this study, it will not affect any other care received at clinic.
• If you say ‘Yes’ now, you can still change your mind later.
• You can quit the study at any time.
• You would not lose benefits or be penalized if you decide not to take part in the study or to quit the study later.”

“You have the option to take part in this research study because you are pregnant. We will enroll women both with and without HIV.”

“If you join this study, you can decide to stop at any time, for any reason. If you decide to stop you would need to talk with site investigators so you leave the study in a safe way.”

“Whether or not you decide to participate in this research study, you can continue to receive your regular mother-child health care at this clinic.”

-Type of specimens and amount to be obtained:
“Blood collection. A study clinician will collect up to 10 mls of blood (teaspoon) from you during enrollment in pregnancy, 6 weeks, and 12 months postpartum. We will also collect up to 5 mls of blood (teaspoon) from your infant at 6 weeks and 12 months of age which will be tested for MTB infection. We will draw an additional 5 mls of blood (teaspoon) form either you or your infant if you or your infant develop active TB for TB infection testing.”

“Breast milk collection. We will collect up to 30 mls of breast milk from you at 6 weeks postpartum.”

- Follow up schedules if applicable/ expected time in study: “We would follow you from the time you enroll in pregnancy until 1 year after you deliver. We would also follow your infant from the time they are born until they turn 1 year of age.

Visit schedule. You would be seen during pregnancy on enrollment and you and your infant would be seen together at 6 weeks, 6 months and 12 months postpartum. These visits are matched with the Kenyan recommended schedule of maternal and pediatric well child/immunization visits.”
- Information on researchers and telephone contacts in case to be contacted

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Department</th>
<th>Telephone Numbers</th>
</tr>
</thead>
<tbody>
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</tr>
</tbody>
</table>

**Emergency telephone number:** Dr. John Kinuthia: +254-722-799-052

- Information on the KNH/OUN/ERC in case they need to contact the committee (phone numbers)

“If I have questions about my rights as a research subject, I can call the Kenyatta National Hospital Ethics and Research Committee, at 2726300 Ext. 44102.

- Any other necessary information about the study:

**Interviews and questionnaires.** During enrollment, we will ask you questions about your health and the health of people in your household. For example, we will ask you about your HIV and TB history, and whether you or your infant have symptoms of TB. If you have symptoms or test results concerning for TB you may be referred to the local TB Control Programme for further evaluation. If you are referred to the TB Control Programme we would like to access your TB evaluation and treatment records.

*These questions will help us understand what may affect a woman’s and her infant’s risk of getting MTB infection. We will keep the answers to these questions private."

**Additional procedures:**

**Tuberculin skin test (TST).** A study clinician will a small needle to put some testing material, called tuberculin, just under your skin on your arm at all of your study visits. We will also do the test for your infant at 12 months of age. We will ask you to return to the clinic in 2-3 days to check the result by measuring if there is a reaction on your skin. TST is a test that is used to diagnose MTB infection, but does not mean you have TB disease. The TST test cannot give you TB, it can only tell us if you have been potentially exposed to TB in the past.

**Future research.** Information that we collect from you, your medical record, and lab results, and specimens may used in future research.

**Missed visit.** It is very important that you come to your scheduled post-partum and infant visits. If you cannot make your appointment, please call the study staff. If you miss your appointment, the study staff will try to contact you. They will do this by trying to call you. They may also talk to the contact people that you list. If they talk to these people, they will not tell them why they are trying to reach you. If study staff cannot reach you, they may visit your household. If this occurs, they will dress in plain clothes and keep the reason for their visit private.”

Additional removal from study procedures:
“The research study clinicians could also decide to take you out of this study. This might happen if we find out that it is not safe for you to continue in the study. It may also happen if you cannot come to enough of the study visits. If we ask you to leave the study we would always explain why and this would not affect other care received at the facility in any way.”

Funding of study:

“The study team and/or the University of Washington and Kenyatta National Hospital are receiving financial support from the National Institutes of Health in the United States”.

Confidentiality:

“We will keep your identity as a research subject confidential. All of your and our infants test results, medical records, and answers to questions will be kept private. No identifying information of any kind will be released to any other person or agency that is not working on this study, without your permission in writing. We will not publish or discuss in public anything that could identify you. Any specimens you provide, and your medical information will be identified by a code number. All of your information, including the link between your name and code number will be kept in a secure location at the clinic only. Once the study is completed, we will maintain the link for 6 years. After this time we will remove your name and all identifying information from the study files. The study team may share identifiable information about you in the case the study team becomes aware of possible harm to yourself or others.

Although we will make every effort to keep your information confidential, no system for protecting your information can be completely secure. It is still possible that someone could find out you were in this study and could find out information about you. Government or university staff may review studies such as this one to make sure they are being done safely and legally. If a review of this study takes place, your records may be examined. The reviewers will protect your privacy. The study records will not be used to put you at legal risk of harm.

Study records may be reviewed by:

- University of Washington, including the Institutional Review Board
- Kenyatta National Hospital and University of Nairobi, including the Ethics and Research Committee
- US National Institutes of Health

- Research-related injury:

“If you think you or your infant has a medical problem or illness related to this research, contact Dr. John Kinuthia: +254-722-799-052 right away. He will treat you or refer you for treatment. If you or your child is injured as a result of being in the study, you will be offered free care at the study clinic. If you require medical care that the study clinic cannot provide, we will refer you to the appropriate organizations to receive care for the injury. The costs of the treatment may be billed to you or the National Hospital Insurance Fund (NHIF) just like other medical costs, or it may be covered by the UW’s discretionary Human Subject’s Assistance Program (HSAP) depending on a number of factors. The researcher may request HSAP coverage by following established procedures. If you wish to request HSAP coverage yourself, you may contact the researchers listed on the first page, or the UW Human Subjects Division at hsdinfo@uw.edu or +1-206-543-0098. Ask the researchers if you would like information about the limits and conditions of the HSAP. The UW does not normally provide any other form of compensation for Injury. However, the law may allow you to seek payment for injury-related expenses if they are caused by malpractice or the fault of the researchers. You do not give up any legal rights by signing this consent form.”
Possible storage of specimen for further analysis with the permission from the KNH/UON/ERC:

“We would like to save your samples at the KEMRI/CDC, University of Nairobi, the University of Washington, Emory University, or the Fred Hutchinson Cancer Research Center for future HIV and/or TB related research and maternal and infant health. This may include testing for other factors which may affect whether a person is more or less likely to get infections, or things that may affect infant and maternal health (mother's health during postpartum period with special emphasis on HIV-related illnesses, infant health with special emphasis on HIV-exposure, and TB exposure).

Information we get from you, and your samples, may be shared with other investigators studying HIV, TB, or mother and child health. We will not share your name or any identifying information with them. An Institutional Review Board or Independent Ethics Committee, which looks at study application to ensure the safety and rights of research participants, must approve future research studies in which we will use your or your baby’s samples to obtain information about both of you. Permission from the University of Nairobi’s Ethics Committee will be sought before any of these samples are used for future research. These tests are for research and are not useful for your or your baby’s clinical care. Before your samples or your baby’s samples leave the clinic, they will be assigned a code and your name or your baby’s name will not be on them. We will store these samples for ten years after completion of the study. Storage of samples past this time period will only occur with approval from an Institutional Review Board and Ethics Committee.

If you do not want to have your or your baby’s samples saved for future research, you can still be in this study and your or your baby’s samples will be destroyed once testing for the study is completed. If you agree to store your or your baby’s samples now, but change your mind before the end of the study, let the study staff know and we will make sure that your or your baby’s samples do not get stored for future research. We will not sell your or your baby’s samples. Tests done on your or your baby’s samples may lead to a new invention or discovery. We have no plans to share any money or other benefits resulting from any potential invention or discovery with you.”
12) DATA MANAGEMENT AND STATISTICAL ANALYSIS PLANS:

Data management plan

Collection and storage of study data. On enrollment, a study chart will be compiled that will contain signed consent forms. With the exception of the locator form and data abstraction from medical records, which will use a paper-based CRF, all other data will be entered directly into password protected tablets using the secure password protected RedCap database sponsored by the UW Institute of Translational Health Science. All data in the database is de-identified and coded by de-identified study ID. The mobile RedCap application allows for data entry onto a tablet without requiring an internet connection at the time of data collection. At the end of each day, data from the table is uploaded to the web-based RedCap online database.

Data will be entered directly by study staff, and the data manager will be responsible for daily upload of data to the online RedCap database. The data manager will discuss each day with the study staff if there are any issues with data entry so that any data entry error is corrected quickly.

The locator form is stored in a locked file cabinet at the study site. The de-identified paper-based forms used only for data abstraction for medical records are de-identified and kept in patient files in a locked file cabinet. Participant files will be accessible only to researchers. Patient identifier and locator information will be kept securely under lock and key.

All laboratory results are de-identified using Study ID. De-identified IGRA QFT-Plus results performed at the KEMRI/CDC lab will be provided in electronic form to be uploaded directly into the RedCap database. Additionally de-identified scanned paper copies of the results will be provided by KEMRI/CDC will be sent directly to the study data clerk from the laboratory to confirm results in the electronic form are correct.

Data checking and validation. The database will be designed with variable range limits to minimize the possibility of inaccurate entries. Correct data entry will be validated by line-listing entered data against printed clinical files.

Monitoring of study protocol and clinical care. The data manager will oversee the generation of weekly reports that will summarize the number of patients seen, medical complications, laboratory results, and specimen collection. These weekly reports of de-identified data will ensure quality assurance for data. This will enable us to ensure that the study is running according to protocol, and to share the data between study investigators in Kisumu, Nairobi, and Seattle.

Statistical analyses

AIM 1 & 2 Outcomes and Analyses:

Aim 1 primary outcome: We will compare the incidence of maternal Mtb infection, defined by positive IGRA conversion between HIV-infected and HIV-uninfected peripartum women. Women with positive IGRA at enrollment or who develop active TB will be excluded from the Aim 1 incidence analysis.

Aim 2 primary outcome: We will compare the prevalence of infant Mtb infection, defined by positive IGRA at 6 weeks or 12 months, between HIV-exposed and unexposed children. Infants found to have active TB will be excluded from the Aim 2 primary analyses.
Additional Aim 1 & 2 primary outcome: We will identify maternal cofactors associated with maternal incident Mtb infection, and maternal and infant cofactors associated with infant Mtb prevalent infection (including age, gestational age, ART, others listed Table 7).

Aim 1 primary analyses: We will use generalized estimating equation (GEE) models with a log link and independent correlation structure to compare the primary outcome of incident maternal Mtb infection incidence between the two maternal cohorts. Potential cofactors for maternal Mtb incidence will be evaluated using multivariable GEE models.

Aim 2 primary analyses: We will use Chi square ($\chi^2$) tests to compare prevalence of infant Mtb infection at 6 weeks and 12 months between HIV-exposed and unexposed infants. Cofactors for infant Mtb will be evaluated using multivariable logistic regression models. Cofactor analysis will include maternal and infant infection status and will be stratified by maternal and infant HIV status to assess any effect modification of HIV infection.

Aim 1 & 2 secondary outcomes #1: We will compare the incidence of a combined endpoint of TB and death between HIV-infected and uninfected peripartum women, and HIV-exposed and unexposed infants using methods described in above Aim 1 & 2 primary analysis.

Aim 1 & 2 secondary outcomes #2: We will compare median magnitude of maternal IGRA responses between HIV-infected and uninfected pregnant women, and between HIV-exposed and unexposed infants using non-parametric tests among IGRA converters and non-converters. Among women with positive baseline IGRA, serial data from 6 weeks and 12 months will be available to estimate stability of IGRA results and reversion. Sensitivity analyses will be performed to estimate incidence using the subset with evidence of persistent detection or above a specified higher threshold level of detection. These data can inform new models to estimate Aim 1 incidence data.

AIM 3 Outcomes and Analyses:

Aim 3 primary outcome & analyses: We will estimate the degree of IGRA/TST agreement at different peripartum stages, using Cohen’s kappa ($\kappa$) coefficient where $\kappa > 0.75$ represents excellent agreement, $\kappa = 0.4 - 0.75$ fair to good agreement, and $\kappa < 0.4$ poor agreement. We will compare the proportion of positive tests at each peripartum stage between HIV-infected and HIV-uninfected women using $\chi^2$ testing. Potential cofactors for positive tests and discordant TST/IGRA (i.e. TST+/IGRA-, TST-/IGRA+) will be evaluated by with multivariable logistic regression models.

Aim 3 secondary outcome & analyses: We will estimate the proportion of reversions for both TST and IGRA, defined as a positive test followed by a negative test. The total proportion of reversion among HIV-infected and uninfected women will be compared by $\chi^2$.

13) STUDY LIMITATIONS AND HOW TO MINIMIZE THEM:

Aims 1 & 2: Limitations/Alternative Approaches: Our sample size and power calculations are based on previous Mtb infection risk estimates in HIV-infected peripartum women and their infants. Total number of incident maternal and infant Mtb infections will limit our power to discern potential cofactors; with only cofactors with appreciable increased risk detected. With fewer outcomes (due to attrition, etc.) minimal detectable difference in proportions will increase. We
expect <10% lost to follow-up based on recent studies. This exploration provides an opportunity to define potentially influential risk factors important for defining clinical suspicion, vigilance, or preventive testing/screening approaches. We anticipate data from Aims 1 & 2 will inform larger Mtb infection correlate studies in HIV-infected women and their children which could evaluate relevant or potential cofactors in more detail. Routine TB culture will not be performed, and subclinical TB is possible. All women and infants will be closely monitored for TB symptoms at each study visit. Symptomatic participants will be referred to the TB program for evaluation per national guidelines, which includes Xpert (rapid TB PCR) as first line for HIV-infected individuals. Longitudinal follow-up (~15 months for mothers, 12 months for infants) will aid in TB disease identification. We have used maternal and infant IGRA positivity estimates, based on TB-SPOT.TB IGRA on cryopreserved specimens in a prior PMTCT cohort. New cohorts may have different Mtb infection prevalence/incidence. Maternal PMTCT regimens (Option B+ maternal ART, infant nevirapine) differ from historical cohorts (short-course zidovudine) which may modify maternal and infant Mtb infection risk. To address reliability and reproducibility of low-level IGRA QFT-Plus positive results (≥0.35 and <0.60 IU/ml), we will perform secondary analyses and compare our results with two definitions of positive QFT (≥0.35 manufacture’s cut-off vs. ≥0.60 IU/ml). Women may receive empiric IPT within the programmatic setting irrespective of TST/IGRA results. Data from TB contact tracing studies and controlled trials of IPT indicate IGRA response is retained after IPT.73,74

Aim 3: Limitations/Alternative Approaches: The total number of positive and discordant maternal TST/IGRA will limit our statistical power to discern potential cofactors. Positive TST may reflect boosting of response with serial testing, or to previous BCG or NTM exposure as opposed to Mtb infection.75 Most boosting occurs 1-5 weeks after testing.75 In our study, testing will occur at longer intervals therefore TST conversion will less likely be due to boosting. IGRA are less effected by boosting, but may occur due to previous TST. Although a single cutoff is used to identify positive IGRA, we will perform analyses for discordance using multiple IGRA cut-offs. Women may receive empiric IPT within programmatic settings irrespective of TST/IGRA results. TB contact tracing and controlled IPT trials indicate IGRA response is retained after IPT.73,74

14) TIMELINE / TIME FRAME:
Enrollment will start in Year 1 and continue until the beginning of Year 5. A study time-line is outlined below.

Table 8: Timeline

<table>
<thead>
<tr>
<th></th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>Year 5</th>
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<tr>
<td>Protocol/CRF development</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>1</td>
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<tr>
<td>Ethical approval</td>
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<td>Staff hiring/training</td>
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<td>Recruitment/ follow-up</td>
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<td>Data analysis</td>
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<tr>
<td>Manuscript prep/submission</td>
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<tr>
<td>Results dissemination</td>
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Protocol v1.3 February 18, 2018
15) REFERENCES

16) BUDGET (total budget period)
This proposal is part of an NIH K23 Mentored Patient-Oriented Research Career Development Award. The budget for the grant which supports staff, equipment, and supplies for the research proposed in this protocol is below.

A) PERSONNEL- SALARIES AND DISBURSEMENTS: $103,900
B) PATIENT COSTS: $1,000
C) SUPPLIES AND EQUIPMENT: $78,500
D) ANIMAL ACQUISITION: NOT APPLICABLE
E) TRAVEL AND ACCOMODATION: $37,875
F) TRANSPORT (VEHICLES, REPAIR, FUEL): $4,750
G) OPERATING EXPENSES (POSTAGE, REPORT WRITING ETC): $1,450
H) CONSULTANCY IF APPLICABLE: NOT APPLICABLE
I) MISCELLANEOUS: $16,927
J) CONTINGENCY (%): NOT APPLICABLE

17) APPENDICES/ATTACHMENTS
I) REFERENCE LIST OF ABBREVIATIONS
II) PROTECTION OF HUMAN SUBJECTS
### APPENDIX I List of Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>AFB</td>
<td>Acid-fast bacilli</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
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<tr>
<td>ANC</td>
<td>Antenatal care</td>
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<tr>
<td>ART</td>
<td>Antiretroviral therapy</td>
</tr>
<tr>
<td>BCG</td>
<td>Bacille Calmette Guerin</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>CXR</td>
<td>chest radiograph</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>ERC</td>
<td>Ethical Review Committee</td>
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<tr>
<td>HEU</td>
<td>HIV-exposed uninfected</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
</tr>
<tr>
<td>IGRA</td>
<td>interferon gamma release assays</td>
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<tr>
<td>IPT</td>
<td>isoniazid preventive therapy</td>
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<tr>
<td>INH</td>
<td>isoniazid</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
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<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
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<td>KNH</td>
<td>Kenyatta National Hospital</td>
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<tr>
<td>KRTC</td>
<td>Kenya Research and Training Center</td>
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<tr>
<td>LTBI</td>
<td>latent tuberculosis infection</td>
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<tr>
<td>LFT</td>
<td>liver function test</td>
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<tr>
<td>MCH</td>
<td>maternal child health</td>
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<tr>
<td>MOH</td>
<td>Ministry of Health</td>
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<tr>
<td>Mtb</td>
<td>Mycobacterium tuberculosis</td>
</tr>
<tr>
<td>PID</td>
<td>Patient identification number</td>
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<tr>
<td>PBMCs</td>
<td>peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PMTCT</td>
<td>prevention of mother to child transmission</td>
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<tr>
<td>PPD</td>
<td>Purified protein derivative</td>
</tr>
<tr>
<td>NIAID</td>
<td>(United States) National Institute of Allergy and Infectious Diseases</td>
</tr>
<tr>
<td>NIH</td>
<td>(United States) National Institutes of Health</td>
</tr>
<tr>
<td>QFT-Plus</td>
<td>Quantiferon Plus</td>
</tr>
<tr>
<td>SAE</td>
<td>serious adverse event</td>
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<tr>
<td>TB</td>
<td>tuberculosis</td>
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<tr>
<td>TST</td>
<td>tuberculin skin test</td>
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<tr>
<td>UoN</td>
<td>University of Nairobi</td>
</tr>
<tr>
<td>UW</td>
<td>University of Washington, Seattle</td>
</tr>
<tr>
<td>VL</td>
<td>Viral load (HIV-1 RNA copies/ml)</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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</tbody>
</table>
APPENDIX II: PROTECTION OF HUMAN SUBJECTS

PROTECTION OF HUMAN SUBJECTS

A. Human Subjects Involvement, Characteristics, and Design

We propose to determine the impact of maternal HIV on the risk of maternal peripartum and infant Mtb infection through prospective parallel longitudinal cohorts of mother-infant pairs using an observational study design. We will compare Mtb infection incidence between HIV-infected and uninfected pregnant women, and Mtb infection prevalence between HIV-exposed and unexposed children. We will also evaluate the effect of maternal HIV status and peripartum stage on the performance of LTBI tests.

A1. Justification

Tuberculosis remains the leading cause of morbidity and mortality among HIV-infected individuals globally. Four out of every five TB cases among people with HIV occur in Africa. Kenya is one of the 22 countries designated by the World Health Organization as a high TB burden country, with an annual TB incidence of 268/100,000. Western Kenya, where this research will take place, has one of the highest antenatal prevalences of HIV in Kenya (19-26%), and 68% of new TB case are among those with HIV. With successful prevention of maternal to child transmission (PMTCT) programs, the population of HIV-exposed but uninfected (HEU) children is growing. These children have increased risk of mortality compared to HIV-unexposed children, and remain at higher risk for TB due to either TB exposure and/or altered immunity associated with maternal HIV.

The aim of this proposed study is to examine the influence of maternal HIV on maternal peripartum Mtb infection incidence and infant Mtb infection prevalence, and compare diagnostic performance of LTBI tests (TST and IGRA) in HIV-infected and HIV-uninfected women in pregnancy and postpartum. TB causes significant morbidity and mortality in women and their children in high TB burden areas. HIV-infected individuals and infants are at high risk of progression from Mtb infection to TB disease. Pregnant and postpartum periods have also been associated with increased risk of TB. We do not know whether maternal HIV increases susceptibility to peripartum maternal or infant Mtb infection. Detecting Mtb infection, particularly recent infection, can identify those individuals who may most likely benefit from preventive therapy.

We are enrolling HIV-infected pregnant women and their children to further understand the risk of Mtb infection, in a population at high risk of progression to active TB with resulting poor maternal and infant outcomes. Comparing Mtb infection incidence in parallel cohorts of HIV-infected and HIV-uninfected peripartum women and Mtb infection prevalence in HIV-exposed and HIV-unexposed infants will allow us to identify whether maternal HIV is associated with increased risk of Mtb infection in these populations.

Additionally, we will identify latent TB diagnostic test characteristics in HIV-infected and HIV-uninfected peripartum women. It is important that this research is performed with peripartum women and their infants as pregnancy and infancy have distinctly different risks of active TB, and potentially Mtb infection. This research could not be conducted in a US setting due to the low burden of HIV/TB and because the results would not be relevant to mothers and their infants in high HIV/TB burden settings.

A2. Study Population

We plan to enroll 200 HIV-infected and 200 HIV-uninfected pregnant women from antenatal care at the Ahero Sub-district and Bondo District Hospitals in the Nyanza region of western Kenya. We will also enroll from Siya Country Referral Hospital, Ranchuonyo sub-County Hospital, Lumumba Health Center, and Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) if
necessary to ensure enrollment goals. **For Aim 1**, we wish to identify the incidence of Mtb infection, therefore we will exclude women found to have prevalent latent TB on enrollment (by IGRA) or active TB, from this analysis. We anticipate approximately 20% of women will have positive IGRA at enrollment based on estimates from our previous TB in Pregnancy studies. We wish to enroll ~165 women in each Aim 1 maternal cohort to account for a conservative estimated 10% lost to follow-up to ensure 150 women with outcomes. For **Aim 2** we will enroll all children born to the women enrolled in the study irrespective of maternal enrollment LTBI testing results. We anticipate there will be approximately 400 infants born to mothers enrolled in the study (~200 HIV-exposed, and 200 HIV-unexposed children). For **Aim 3** we will include mothers irrespective of enrollment LTBI testing results (~200 HIV-infected, and 200 HIV-uninfected peripartum women).

### A3. Sampling plan, recruitment and retention strategies and inclusion/exclusion criteria.

#### Sampling plan
We will consecutively enroll 200 HIV-infected and 200 HIV-uninfected pregnant women in antenatal care at the Ahero Sub-district and Bondo District Hospitals in western Kenya who are interested and willing to participate in the research and their infants (200 HIV-exposed, 200 HIV-unexposed). Participation in the study will involve agreeing to physical exams, surveys and labs/procedure from enrollment in pregnancy to 12 months postpartum (Appendix 5).

#### Eligibility
Women are eligible for the overall study if they are pregnant (between 20-34 weeks gestation) and at least 16 years old, have not been treated for active tuberculosis in the last year, and are willing to return for follow-up visits. Women who are 16 years of age and pregnant are able to provide study consent, as their pregnancy emancipates them for participation in research under Kenyan law.

#### Enrollment and consent
Potential participants will be informed about the study by antenatal clinic staff/nurses and referred to the study staff for more information about the research. Following informed consent and study screening procedures, pregnant women will be offered enrollment into the study. Informed consent will occur in a one-on-one counseling session with a study staff person in either Kiswahili, Dholuo or English according to participant’s preference. A copy of the consent form in the language of preference will be offered to the participant. For women who are not literate, the consent will be read to them in the appropriate language of their choosing with a witness independent of the study present. For women who cannot write, an inked thumbprint will be used in lieu of signature. Women will provide consent for their infants.

#### Procedures
**Maternal cohorts:** Physical exams and sociodemographic and health surveys will be performed on enrollment in pregnancy, 6 weeks, 6 months and 12 months postpartum. Sociodemographic and health surveys will include questions about age, marital status, HIV, TB (including known TB contacts and other potential TB exposures), obstetrical history, educational status, household crowding, and World Health Organization TB symptoms screen (cough, fever, weight loss, night sweats) of both the participant as well as reported household member symptoms, and intercurrent illness. TSTs will be placed and blood will be drawn on enrollment, 6 weeks, and 12 months postpartum. TSTs will be read within 48-96 hours of placement by a study nurse. Breast milk will be collected at 6 weeks postpartum. HIV-uninfected women at enrollment will also be offered HIV testing per national protocols through maternal child health clinic HIV counselors at enrollment into the study, 6 weeks, 6 months and 12 months postpartum.

**Infant cohorts:** Physical exams and sociodemographic and health surveys will be performed on enrollment at 6 weeks of age, 6 and 12 months. Information regarding infant PMTCT prophylaxis, birth weight, BCG vaccination date, intercurrent illnesses, and other immunizations will be
collected on enrollment. At each visit infant examination will include growth measures and a questionnaire addressing infant feeding (including breastfeeding status) and symptoms (including cough, fever, weight loss, failure to thrive) will be performed. Blood will be drawn at 6 weeks and 12 months of age. Due to the high rates of BCG cross-reactivity after recent immunization, we will not perform TSTs in infants until 12 months of age.

Timing of visits is designed to align with routine ante/postnatal care, maternal ART and infant immunization visits.

Retention
Women will be asked to give contact information to study staff as well as contact information for a trusted person who may be able to locate them. Women will use a locator map to describe their primary residence and how to get there. The study staff and the subject will craft a detailed plan for each subject describing how best to contact them and allowing the subject to share any relevant information (for example, if the subject has not disclosed her HIV status or pregnancy). Study staff may contact subjects outside of clinic with their permission, but will never identify themselves by other than a first name and will not leave messages. Study information will not be discussed by telephone. Home visits may also occur with the subject’s permission. At every step of the retention process, discretion and participant confidentiality will be the top priority. Subjects will be reimbursed for their transportation for each visit and all study related services are provided free of charge. Retention in our prior maternal child cohort studies has been high (>90%). Travel reimbursement, and aligning visits with routine medical care (child immunization and maternal ART visits) is likely to contribute to high retention in this proposed study.

A6. Justification for involvement of children and pregnant women (Vulnerable populations)
Please see justification described in A1. above. This research can only be done by enrolling HIV-infected and HIV-uninfected peripartum women and their infants, in order to understand the role of HIV in pregnancy on the risk of maternal and infant MTb infection, as well as to understand the role of pregnancy stage and HIV on latent TB diagnostics.

A7. Assignment to study groups
There is no randomization component to this study. Subjects in both maternal cohorts will have the same study testing, however HIV negative women will be additionally referred for HIV testing during enrollment, and at 6 weeks, 6 months and 12 months postpartum at the onsite MCH clinics. HIV-exposed children will receive HIV PCR testing at 6 weeks, and at 1 year HIV testing will be performed per national guidelines.

A8. Collaborative sites/study location/research site
The proposed study will be conducted in the Nyanza Province of western Kenya at the antenatal, postpartum, and pediatric clinics located at the Ahero Sub-district and Bondo District Hospitals. The region has high antenatal HIV prevalence (19-26%), and high antenatal clinic attendance (~200 new pregnant women per month). These study sites have been involved with numerous UW-associated studies including those focused on HIV and TB in pregnancy over the past 4 years. The study location in western Kenya is important due to the high prevalence of both HIV and TB. This study will provide data which will aid in determining recommendations for TB prevention efforts in maternal and child health settings. Studies of this nature have not been done and are vital to identify those who may most benefit from preventive therapy as well as ascertain the timing and type of recommended latent tuberculosis test. This study is an extension of ongoing research with HIV-infected pregnant women and our research group routinely works with HIV-infected women and their children in Kenya. Our study team members are acutely sensitive to protecting participants’ interests during the conduct of this research. Both HIV-infected and HIV-uninfected women will be enrolled in this study, therefore we do not anticipate that participation in the study will risk revealing a woman’s HIV status to other women in the clinical setting.
Study staff will work in conjunction with antenatal and pediatric staff to identify potential participants. Study staff will help any women or infants with suspected tuberculosis to access care at TB clinics, as well as HIV care clinics if necessary. This insures that care for all women and children in this maternal and child health setting is not compromised by the presence of the study, and should ensure that subjects do not feel pressure to participate in the study to receive antenatal, postnatal, pediatric, TB or HIV-related services. Study staff have been working side by side with clinic staff at these sites for >4 years. Study staff are well trained in recruitment and are knowledgeable in recruitment without persuasion/coercion.

B. Sources of Materials

B1. Biological specimens

Blood will be drawn from patients for TB immunologic studies including whole blood for IGRA and for PBMCs and plasma separation for flow cytometry (mothers (10 ml): pregnancy, 6 weeks postpartum, 1 year postpartum; infants (5 ml): 6 weeks, 1 year of age). HIV-uninfected women will be referred to HIV counselors for HIV testing at 6 weeks, 6 months and 12 months postpartum. HIV exposed infants will be referred for HIV PCR testing at 6 weeks, and at 1 year HIV testing will be performed per national guidelines.

Breast milk (30 mls) will be collected from mothers at 6 weeks postpartum for maternal antibodies to TB.

B2. Data collection

At the enrollment visit we will collect basic demographics and clinical characteristics including age, marital status, obstetrical history, economic status, educational status, and household crowding. Women will be interviewed and their medical chart reviewed in order to collect data regarding HIV status, TB history (including reported TB contacts) as well as demographic information.

Data management

Participant files will be accessible only to researchers and will be stored in a location outside the clinic in a locked office. Study databases will not include patient identifiers. Data will be stored in a secure password-protected online RedCap database sponsored by UW Institute of Translational Health Science. A participant list with patient identification and study ID will be kept in a locked file cabinet. All patient specimens will be marked by a de-identified study ID. Participant files will be accessible only to researchers and will be stored the research office in a location outside the clinic in a locked office. Study databases will not include patient identifiers. Written consent or a witnessed thumbprint if not literate will be obtained from all participants prior to enrollment. Consent will be provided in Kiswahili, Dholuo and English languages.

C. Potential Risks

C1. Confidentiality

The primary risk to the subjects is the potential for loss of confidentiality. Disclosure of HIV status is a non-negligible risk. If the patient’s HIV status was known, this could lead to psychological harm or affect the standing of the subject in the community. We believe the risk of such disclosures is low, however, due to this risk, we will implement the protections to ensure security. Each subject will be given a number upon entry to the study; recorded survey data and study information will include only a study number and will not contain the subjects’ names. A subject could conceivably incur psychological harm or loss of confidentiality through our attempts to call them or reach them through retention protocols. However, we will only call subjects on a number that they have given permission to use, and we will never discuss the subject of the research study in any telephone conversation. If we reach someone other than the participant on the telephone, we will only leave a name and callback number, not disclose information about the purpose for the call or identify association with the research project. As is standard practice in Kenya, health workers sometimes will visit a participant’s home for retention purposes. This is
done with great discretion and the purpose of the home visit is never discussed with anyone other than the study participant. This protocol will be described in detail to subjects at study entry, and subjects may decline to provide contact information for anyone other than themselves. All study personnel have been trained in Good Clinical Practice and confidentiality.

C2. Specimen collection
Risk of pain and bruising from phlebotomy: (Maternal and infant cohorts) Phlebotomy will be performed by nurses trained in optimal phlebotomy practices in both adults and children and will be performed when subjects are sitting or lying down. At each visit where blood is scheduled 10 ml of blood will be collected from mothers, and 5 ml of blood will be collected from infants. We do not anticipate any study-related physical adverse effects to participants.

C3. TST Placement
Risk of pain from TST placement: TST is contraindicated only for persons who have had a severe reaction (e.g., necrosis, blistering, anaphylactic shock, or ulcerations) to a previous TST; this is a rare (<1%) occurrence. TST use is uncommon in the study area in Kenya. Prior to TST placement, subjects will be asked if they have had a severe reaction to TST in the past. TST will not be placed in study participants who have had a previous severe reaction). For mothers TST will be placed at enrollment, 6 weeks and 12 months postpartum regardless of previous positive reaction to TST. For infants TSTs will be placed at 12 months of age. TST will be read at 48-96 hours after placement by study staff.

D. Adequacy of Protection Against Risks
D1. Recruitment and informed consent
Potential subjects recruited for this trial are limited to those persons who are pregnant, ages 16 or older, receiving antenatal services, and their children who are born at the end of the pregnancy during which the subject is enrolled. Potential subjects will have access to all available antenatal, postnatal, pediatric, HIV, and TB services regardless of their decision to participate in the research. Patients who are interested in learning more about the study will be able to complete the informed consent process and the study enrollment visit on the same day. Access to antenatal services or maternal child health services will not be delayed based on inclusion in the study. Once patients consent to study participation, they will be asked to sign an informed consent form. Informed consent will occur in a one-on-one counseling session with a study staff person and will occur in either Kiswahili, Dholuo or English according to participant’s preference. During the informed consent counseling, the counselor will describe the study in detail, review the consent form, including reviewing expected benefits and possible risks of participation, and answer all questions a subject may have. Subjects will be notified that they can withdraw consent for participation in the study at any time. A witnessed thumbprint if not literate will be obtained from all participants prior to enrollment. Mothers will provide consent for their infant’s enrollment.

Alternatives to study participation
If patients are not interested in the study, they will not be enrolled into the study.

D2. Protections against risk
Protecting confidentiality in the research clinic
At the enrollment visit, subjects will be asked questions that include sensitive topics such as HIV status, TB exposure, household symptoms, and pregnancy history. The survey will be administered orally by the study nurse in a private setting. The written responses will go in the study subject’s folder identified only by their study ID number. The subject numbers will be linked to names and demographic information in a separate file to allow linkage with data from the medical record. This file will be stored separately from the patient file. Data entry personnel will only have access the anonymous survey, with the study participant number, not the list linking
subject numbers to names. Only study investigators will have access to the list linking names to subject numbers. All specimens collected from participants will be labeled with subject’s number. The list linking subject numbers to names will be kept in a locked file cabinet in a locked office. De-identified data will be kept on a secure, password and firewall-protected computer that is located in a locked study office near the clinics.

Protecting confidentiality during retention efforts
Our retention protocol has been used in other research studies at the same clinic and is designed carefully and explicitly to protect the privacy of study subjects. All participants in the study will create their own retention plan. They are asked questions such as “If we need to reach you, what is the best way to do it?” which gives them an opportunity to create a plan. An example of such a plan is that some women may prefer study staff to visit their workplace but not their home; some may give permission to try to reach a sister, but not a partner/husband. In this way the retention plan is personalized for each woman’s specific situation. Study staff in charge of retention are highly sensitive to each subject’s privacy and are also part of the plan created to ensure that study participation is safe for the subjects. Regardless of a subject’s preferences, during retention efforts, our study workers will never identify themselves as part of a research project, health care or the health center. They will identify themselves as “Subject’s friend X is trying to reach them” and leave a number to call. In this way subjects can feel confident that none of our study employees will inadvertently disclose protected information or create suspicion in the home.

Procedures to minimize pain and bruising from phlebotomy
Phlebotomy will be performed by nurses trained in optimal phlebotomy practices for both adults and children and will be performed when subjects are sitting or lying down.

Procedures to minimize adverse effects from TST placement
TST placement will be performed by nurses trained in optimal TST placement and will be performed when subjects are sitting or lying down. Prior to TST placement subjects will be asked regarding any previous severe reaction to TST (e.g., necrosis, blistering, anaphylactic shock, or ulcerations). If a subject has had a previous severe reaction to TST, it will not be placed during the course of the study.

Procedures to minimize risk of HIV, vertical transmission of HIV
HIV-uninfected women will be counseled in ways to reduce their risk of HIV acquisition, including condom use. HIV-infected women will be counseled on the prevention of mother to child transmission per Kenyan guidelines. Previously HIV-negative women and HEU infants found to be HIV-infected during the study will be immediately referred to appropriate HIV services. HIV evaluation and treatment is free per Kenyan National HIV guidelines.

Procedures to minimize risk of progression from latent to active TB
HIV-infected women with evidence of latent TB, or development of new Mtb infection (by either positive TST or IGRA) during the study will be referred to the HIV comprehensive care clinic on site to receive isoniazid preventive therapy per Kenyan guidelines. Children identified as having close contact with a person with TB will also be referred to the TB program clinic for isoniazid preventive therapy per Kenyan guidelines. All subjects identified as having potential active TB will be referred to the TB program for further investigation as well as treatment as required per Kenyan National TB guidelines including CXR and Xpert as necessary. Isoniazid prevention therapy and TB evaluation and treatment is free per Kenyan National TB guidelines.

Procedures to minimize risk of pregnancy complications
Women with high-risk pregnancies, or risks of complications will be referred to deliver at their closest MCH-linked maternity center (Bondo District Hospital or Ahero sub-District Hospital). Women or children developing other unexpected health problems that are not addressable within the study clinic will be referred to the appropriate clinic within the hospital.
Procedures to ensure newly diagnosed HIV-infected women receive HIV care
Enrolled HIV-uninfected pregnant women will be referred for HIV tests through MCH trained HIV counselors at enrollment, 6 weeks, 6 months and 12 months postpartum. If any women are found to be HIV-infected they will be referred for HIV care (including prevention of mother to child transmission of HIV services) through the HIV care clinic.

Procedures to ensure newly diagnosed HIV-infected children receive HIV care
Enrolled HIV-unexposed infants referred for HIV tests through MCH trained HIV counselors at 6 weeks and 12 months of age. If any of these children are found to be HIV-infected they will be referred immediately for HIV care through the Pediatric HIV care clinic.

E. Potential Benefits of the Proposed Research to Human Subjects and Others
The proposed research will provide important information regarding the extent that HIV in pregnancy increases susceptibility to Mtb infection in peripartum women and their infants, as well as the role of HIV and peripartum stage on the performance of latent TB diagnostics. The risks to the individual subjects are low. Benefits to participants include close monitoring of TB symptoms which will prompt referral to appropriate screening and treatment facilities and serial testing of HIV which may identify newly infected women and their infants. The subjects from the HIV-infected maternal cohort with evidence of Mtb infection, as well as infants found to have TB contacts, will benefit from referral for isoniazid prophylaxis, as well as from focused questioning regarding TB risk factors and prompt referral to the TB program if concern for active TB arises. The information from the results of this study could benefit other peripartum women and their children in areas of high HIV/TB burden.

F. Importance of the Knowledge to be Gained
The proposed research will help define the effects of HIV in pregnancy on the risk of MTb infection in peripartum women and their infants. For the HIV-uninfected mother cohort, the research will further characterize latent TB test characteristics in pregnancy and evaluate MTb infection risk in their infants in a high TB burden setting. As millions of peripartum women and their children live in areas of high HIV and TB burden, knowledge derived from this study could improve TB prevention strategies within maternal and child health settings throughout sub-Saharan Africa, as well as in other high HIV/TB burden areas.

G. Data and Safety Monitoring Plan
The principal investigator (PI) will take responsibility for data safety and monitoring as part of the general oversight and scientific leadership of the study. Commensurate to the non-interventional nature of the study, as well as low risk to participants, we do not plan to have an independent data and safety monitoring board. The PI will report all unanticipated problems involving risks to the subjects or others to the University of Washington IRB and the Kenyatta National Hospital Ethical Research Committee in writing within 48 hours of the occurrence of the adverse event. The PI will also submit a research summary, safety summary, and adverse event information to the IRB and ERC annually.

H. ClinicalTrials.gov
This trial does not fit criteria for mandatory registration with ClinicalTrials.gov.