

HIVNET 024

**PHASE III TRIAL OF ANTIBIOTICS TO REDUCE
CHORIOAMNIONITIS-RELATED PERINATAL HIV TRANSMISSION**

A Study of the HIVNET Group

Sponsored by:

**The National Institute of Allergy and Infectious Diseases and the National Institutes of
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TABLE OF CONTENTS

ACRONYMS		vii
SCHEMA		viii
1.0	INTRODUCTION	1
1.1	<u>BACKGROUND</u>	1
1.2	<u>RATIONALE</u>	2
2.0	STUDY OBJECTIVES	4
2.1	<u>PRIMARY</u>	4
2.2	<u>SECONDARY</u>	4
3.0	STUDY DESIGN	6
3.1	<u>SELECTION AND ENROLLMENT OF SUBJECTS</u>	7
3.2	<u>CO-ENROLLMENT GUIDELINES</u>	8
4.0	CLINICAL AND LABORATORY EVALUATIONS	8
4.1	<u>PRE-ENTRY/ENTRY EVALUATIONS</u>	8
4.2	<u>EVALUATIONS DURING PREGNANCY AND LABOR</u>	9
4.3	<u>EVALUATIONS AT THE TIME OF PERMANENT TREATMENT DISCONTINUATION</u>	13
4.4	<u>POST-TREATMENT EVALUATIONS</u>	14
5.0	DATA COLLECTION AND MONITORING AND ADVERSE EXPERIENCE REPORTING	16
5.1	<u>RECORDS TO BE KEPT</u>	16
5.2	<u>DATA MANAGEMENT AND DOCUMENTATION GUIDELINES</u>	16
5.3	<u>REGIONAL MONITORING</u>	16
5.4	<u>ADVERSE EXPERIENCE REPORTING</u>	17
6.0	STUDY TREATMENT	17
6.1	<u>DRUG REGIMENS, ADMINISTRATION AND DURATION</u>	17
6.2	<u>DRUG FORMULATION</u>	18
6.3	<u>DRUG SUPPLY, DISTRIBUTION AND PHARMACY</u>	18
6.4	<u>CONCOMITANT MEDICATIONS</u>	18
6.5	<u>TOXICITY MANAGEMENT</u>	18
6.6	<u>CRITERIA FOR TREATMENT DISCONTINUATION</u>	18
6.7	<u>OTHER INFECTIONS</u>	19
7.0	STATISTICAL CONSIDERATIONS	19
7.1	<u>GENERAL DESIGN ISSUES</u>	19
7.2	<u>ENDPOINTS</u>	19
7.3	<u>RANDOMIZATION AND BLINDING PROCEDURES</u>	20
7.4	<u>SAMPLE SIZE AND ACCRUAL</u>	21
7.5	<u>MONITORING AND ANALYSIS</u>	21
8.0	HUMAN SUBJECTS	23
8.1	<u>INSTITUTIONAL REVIEW BOARD (IRB) REVIEW AND INFORMED CONSENT</u>	23
8.2	<u>SUBJECT CONFIDENTIALITY</u>	24
8.3	<u>STUDY DISCONTINUATION</u>	24
9.0	PUBLICATION OF RESEARCH FINDINGS	24

10.0	BIOHAZARD CONTAINMENT	24
11.0	REFERENCES	25
APPENDIX I	SCHEDULE OF EVALUATIONS	28
APPENDIX II.	DIVISION OF AIDS TOXICITY TABLE FOR GRADING SEVERITY OF PEDIATRIC (< 3 MONTHS OF AGE) ADVERSE EXPERIENCE	30
APPENDIX III.	ADVERSE EXPERIENCE REPORTING GUIDELINES	38
APPENDIX IV.	SAMPLE INFORMED CONSENTS	42
APPENDIX V.	“CHORIODECIDUAL INFLAMMATION: A POTENTIALLY PREVENTABLE CAUSE OF PERINATAL HIV TRANSMISSION?	53
APPENDIX VI.	EVALUATION OF PLACENTA.....	69
APPENDIX VII.	TIMELINE	70

*The format for this protocol is that recommended by the HIVNET Perinatal Working Group.

ACRONYMS

AE	adverse experience
AIDS	Acquired Immune Deficiency Syndrome
AZT	zidovudine
BPM	beats per minute
BV	bacterial vaginosis
CRF	case report form
DAIDS	Division of AIDS
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
EIA	enzyme immunoassay
FFN	fetal fibronectin
FHI	Family Health International
GC	gonorrhea culture
HIV	human immunodeficiency virus
HIVNET	HIV Network for Prevention Trials
IRB	institutional review board
IU	international units
MCT	maternal-child transmission
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
PAVE	Preparation for AIDS Vaccine Evaluation
PCR	polymerase chain reaction
PRAB	Pharmaceutical and Regulatory Affairs Branch, DAIDS
PROM	premature rupture of membranes
QECH	Queen Elizabeth Central Hospital, Blantyre, Malawi
RNA	ribonucleic acid
ROM	rupture of membranes
RPR	rapid plasma reagin (test for syphilis)
RT-PCR	reverse transcriptase polymerase chain reaction
SAE	serious adverse experience
STD	sexually transmitted disease
TID	three times a day
TPHA	<i>Treponema pallidum</i> hemagglutination
USAID	United States Agency for International Development

SCHEMA

PHASE III TRIAL OF ANTIBIOTICS TO REDUCE CHORIOAMNIONITIS-RELATED PERINATAL HIV TRANSMISSION

- DESIGN:** This will be a randomized, double-blinded, controlled Phase III trial of antibiotics to prevent chorioamnionitis-associated perinatal HIV transmission. Using a simple 2-arm design, half the subjects will receive two courses of antibiotics, with the control subjects receiving two courses of a placebo.
- SAMPLE SIZE:** Using a target of 25% reduction in HIV transmission and with 90% power, the sample size will be 2100 HIV-positive pregnant women. In order to prevent stigmatization and to investigate the impact of antibiotics on HIV-negative women in this setting, 600 additional HIV-negative women will be enrolled and treated indistinguishably from those who are HIV-positive.
- POPULATION:** This study will be conducted at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi and four urban clinics in Lusaka, Zambia. Women will be recruited from the antenatal clinics. After obtaining informed consent, HIV seropositive (and seronegative) women will be enrolled, followed during pregnancy, and requested to deliver in the hospital or clinics. Women and infants will attend postnatal follow-up visits in designated clinics at QECH and in Lusaka.
- REGIMEN:** At 20 – 24 weeks, women randomized to receive antibiotics will receive Metronidazole 250 mg three times a day (TID) and erythromycin 250 mg orally TID for 7 days. Women randomized to the control group will receive identically appearing placebos. With the onset of contractions and/or premature rupture of membranes (PROM), study participants will initiate a second oral course of antibiotics consisting of Metronidazole 250 mg and ampicillin 500 mg or placebo every 4 hours, continuing after delivery TID until the course is completed.
- OBJECTIVE:** To determine if low-cost antibiotic treatment given twice during pregnancy (total cost less than \$5.00) aimed at reducing chronic and acute chorioamnionitis will reduce perinatal HIV transmission.

1.0 INTRODUCTION

1.1 Background

Obstetric risk factors for HIV maternal-child transmission (MCT) include preterm birth, prolonged rupture of the membranes, and both clinical and histologic chorioamnionitis. Each of these risk factors for HIV MCT may operate through a common pathway in which HIV-infected maternal white cells enter the amniotic fluid following bacterial infections of the placental membranes and result in both “histologic chorioamnionitis” and perinatal HIV transmission.

The relationship between chorioamnionitis and preterm birth has been investigated for a number of years.¹⁻¹⁰ Findings include the observation that a substantial number of preterm births are associated with and likely caused by chorioamnionitis, with this sub-clinical infection preceding labor or spontaneous rupture of membranes. The earlier the gestational age at onset of spontaneous labor, the more likely it is caused by chorioamnionitis, with histologic chorioamnionitis found in more than 80% of the spontaneous preterm births less than 30 weeks gestation. The organisms associated with this intrauterine infection in every population studied to date are of relatively low virulence and include *Ureaplasma*, *Mycoplasma*, *Bacteroides*, *Mobiluncus*, and *Gardnerella*.^{1,4}

Documented intrauterine infections present at 16 weeks gestational age or earlier are associated with preterm labor as late as 32 to 34 weeks. Bacterial vaginosis (BV), an overgrowth of these organisms in the vagina, is associated with and probably a marker for chorioamnionitis. In more than 20 studies to date, BV predicts a two-fold or more increased risk of spontaneous preterm labor.¹ A randomized trial of antibiotics (Metronidazole/erythromycin) in women at risk for spontaneous preterm birth who also had BV resulted in a substantial reduction in the rate of preterm birth in that population.⁷ Other studies have confirmed this result. Also, investigators in Malawi have demonstrated that the presence of BV is not only a risk factor for male-female HIV transmission, but for MCT as well. Preliminary results from Malawi show a 2-fold increase (28% vs. 14%) in HIV MCT in BV positive vs. BV negative women.^{11, 12}

A better predictor of spontaneous preterm birth than BV is fetal fibronectin (FFN), a placental membrane derived protein which when found in the vagina after 20 weeks gestation is the most potent predictor (odds ratio = 60) of spontaneous preterm birth yet described.¹³ Fetal fibronectin, when found in the vagina at 20 to 24 weeks gestational age, is a strong predictor of neonatal sepsis (odds ratio = 6) and a very potent predictor of clinical chorioamnionitis (odds ratio of 20), and is nearly always associated with histologic chorioamnionitis at delivery.¹⁴ For reasons of both cost and practicality, it is not expected that fetal fibronectin will be routinely used in subSaharan Africa. However, if a positive fetal fibronectin is strongly associated with HIV MCT, our understanding of the mechanism will be greatly increased.

The findings described above are pertinent to understanding at least a part of the perinatal transmission of HIV, especially because perinatal HIV transmission is more common in preterm infants and there is now evidence that sub-clinical chorioamnionitis is a substantial risk factor for MCT.¹⁵ For this study, the primary hypothesis is that early and appropriate treatment of sub-clinical chorioamnionitis prior to the onset of spontaneous preterm labor, and/or antibiotic treatment during labor to prevent PROM-associated-chorioamnionitis will reduce the risk of perinatal HIV transmission. For further discussion of this hypothesis, see the paper attached in Appendix V (in press, The Lancet).

1.2 Rationale

This intervention to reduce HIV MCT is chosen for evaluation because:

1. It has biologic plausibility for reducing HIV MCT.
2. It is very low in cost compared to antiviral therapy (i.e., a full course of Metronidazole in Zambia or Malawi is \$0.60, erythromycin \$1.50, and ampicillin \$2.50. The two courses of antibiotic treatment will be approximately \$4.30).
3. If, ultimately, antiretroviral agents are used only near delivery, it is not likely that they will reduce the portion of HIV MCT occurring earlier in pregnancy. Antibiotic treatment as described in this protocol has biologic plausibility for reducing antepartum as well as peripartum HIV MCT and for achieving additional reductions in HIV MCT over and above that achieved with short course AZT alone, if that treatment is available.

The specific antibiotics to be used prenatally include Metronidazole and erythromycin. The rationale for choosing these two antibiotics includes: 1) the organisms discussed above are generally sensitive to one or both of these antibiotics; 2) only Metronidazole and/or Metronidazole and erythromycin have been shown to be associated with a reduction in spontaneous preterm birth; 3) Metronidazole has been used during pregnancy in the United States and elsewhere for a number of years and is safe, especially in the second trimester. Erythromycin and ampicillin are also safe during pregnancy.

An initial treatment course of Metronidazole and erythromycin will be given orally three times per day for seven days at 20-24 weeks gestation. This course was chosen for the following reasons: 1) in the second trimester women are being treated for an intrauterine infection and not solely bacterial vaginosis, and 2) the two trials in which a reduction in prematurity has been achieved used a prolonged course of antibiotics (one week or more) in the second trimester.

In labor and with ruptured membranes, since Group B Streptococcus is often the offending organism and rapid antibiotic entrance into the amniotic fluid is an important issue, Metronidazole 250 mg in combination with oral ampicillin 500 mg will be administered orally every 4 hours to prevent the acute chorioamnionitis

associated with membrane rupture. Both of these drugs achieve satisfactory serum and amniotic fluid levels within 1-2 hours after oral administration.

Organisms associated with both BV and chorioamnionitis have been similar whether studied in the U.S., Europe, Asia or South Africa, and have not differed substantially between black and white women in the U.S, Great Britain or South Africa.^{1,4,9} A randomized trial in South Africa showed a reduction in preterm birth in women in early preterm labor with intact membranes who used Metronidazole and ampicillin.⁹ Therefore, although most studies dealing with infection and preterm birth have been performed elsewhere, it is very likely that they are applicable to subSaharan Africa. Even if this study only demonstrates that the antibiotic strategy employed in this protocol reduces preterm births in subSaharan Africa, this result in itself will be of major importance to the women of these countries.

Currently, there is little consensus in the United States and other countries about the value of routinely screening for infection and treating pregnant women with antibiotics either prior to or during labor to prevent preterm birth. For example, while there is some evidence that treating women who had a previous preterm birth who were BV positive reduced preterm birth, there are several recent reports which suggest no benefit when large populations of women are screened and treated.¹⁶ In fact, recently analyzed data from a large -scale NIH trial suggested no reduction in preterm birth using this approach¹⁷. In view of these findings, no major U.S. organization has yet made a recommendation for widespread screening for and treatment of bacterial vaginosis during pregnancy. Since this practice is not routine in the U.S. and in fact is under study in a number of U.S. locations, the investigators believe it is also appropriate to study antibiotic use during pregnancy using placebo controls in an African setting.

If antibiotic treatment aimed at reducing chorioamnionitis significantly reduces transmission, the study results as well as information regarding the cost of implementing the intervention will be provided to appropriate Ministry of Health officials and other policy makers in both Zambia and Malawi. Ministry of Health officials in both countries are aware of and are enthusiastic about this study because it offers the potential for a low-cost reduction in perinatal HIV transmission. While there are no formal guarantees of nationwide adoption, the introduction of this strategy will be evaluated in each location. In addition, the investigators have already had discussions with USAID officials about funding the use of these medications in demonstration projects in these countries, if the trial is successful.

1.2.1 Antiretroviral considerations

AZT is not currently available in Malawi or Zambia for routine antenatal use for HIV infected women, and there are no immediate plans to initiate use of AZT in these countries. In spite of the success of a shorter AZT

regimen in Thailand, the cost is high (about \$80 per woman). Based on the assessment of the in-country investigators in both countries, it is unlikely that antiretroviral drugs will be available soon, or be included in the essential drug list of either of these countries. If AZT becomes the standard treatment for pregnant women in either country, the proposed study would incorporate this regimen into the study design. At present, we would expect that if AZT became routinely available in Zambia and Malawi, each study participant would receive the medication routinely as directed by the Ministry of Health. Assuming that the Thai regimen or something similar is used, with a 50% reduction in transmission, the study would still be feasible but with a larger (approximately double) sample size. Specifically, if AZT became available, every woman would receive the AZT and the antibiotic regimen as proposed would be tested versus a placebo regimen.

As recommended in a recent meeting sponsored by the Pediatric AIDS Foundation and Emory University, “there is a need to evaluate simple and feasible interventions other than antiretroviral agents for their efficacy in diminishing HIV MCT in developing nations”. They also noted that “in the study of non-antiretroviral interventions (e.g. vaginal lavage or antibiotic treatment), a “no drug” intervention control design may be justified ethically in a setting where antiretroviral therapies are not generally available to any individuals in the community”. They also recommend that successful simple measures that are affordable and sustainable should be included as part of the standard care in developing countries with limited resources. Therefore, recently proven beneficial therapies such as syphilis testing and treatment and multi-vitamin supplementation during pregnancy will be provided. All women will receive a multi-vitamin supplement based on the results of a study from Tanzania.¹⁸ The supplement is in addition to routine antenatal care, which consists of iron and folic acid for all pregnant women.

2.0 STUDY OBJECTIVES

2.1 Primary

- To determine if antibiotic treatment aimed at reducing chronic and acute chorioamnionitis will reduce HIV MCT.

2.2 Secondary

- To determine if antibiotic treatment reduces preterm birth and maternal/infant morbidity and mortality.

- To determine if antibiotic treatment reduces HIV MCT in women with BV or a positive FFN.
- To determine if the reduction in HIV MCT potentially achieved by antibiotics is associated with a reduction in histologic chorioamnionitis, preterm birth, or vaginal viral load.
- In HIV-negative women, to determine if the antibiotic regimen employed in this study is associated with an improvement in birthweight and other perinatal outcomes.
- To determine the correlation between vaginal pH, the whiff test, a non-specific vaginal discharge and clue cells on a wet prep with a Gram stain diagnosis of BV, and to determine the sensitivity, specificity, positive and negative predictive values of these tests for diagnosing BV and predicting HIV MCT.

The secondary end points include preterm birth and infant and maternal morbidity and mortality. In developing countries, most preterm infants die. Even in term infants, mortality is often associated with infections acquired during childbirth. There is substantial data emerging from many sources that preterm birth is associated with a chronic but asymptomatic chorioamnionitis, and that chorioamnionitis at delivery is associated with neonatal infection, morbidity and mortality. It is, therefore, reasonable to hypothesize that antibiotic prophylaxis may reduce preterm birth and its associated morbidity and mortality, as well as the infectious morbidity and mortality associated with chorioamnionitis acquired near delivery. Maternal morbidity and mortality may also be reduced. This study will allow determination in both HIV positive and HIV negative women of whether perinatal morbidity and mortality may be reduced with prophylactic antibiotics.

The investigators are also interested in determining whether or not bacterial vaginosis and fetal fibronectin predict HIV MCT, and if women with these findings are more or less responsive to antibiotics for both the primary and secondary outcomes. The investigators expect the prevalence of BV in this population to be 30-50%. The prevalence of women testing positive for fetal fibronectin is expected to be 6-8%. Both of these “vaginal findings” have been linked to chorioamnionitis. There is therefore reason to believe that their presence may be related to both the primary and secondary outcomes. In this study, if antibiotics work to reduce HIV MCT, the cause of the reduction will be of interest. By obtaining the above information, examining the placenta for evidence of histologic chorioamnionitis in the treated women and controls, measuring vaginal viral load and determining if the antibiotics reduced the HIV MCT associated with preterm birth, a better understanding of the relationship between HIV MCT and antibiotics will be achieved.

Finally, although there is preliminary evidence that the presence of BV may be associated with HIV MCT, even this conclusion is uncertain. Furthermore, since BV may be diagnosed in many different ways, and it not clear which, if any, of

the many diagnostic tests predict HIV MCT, correlating this information with the various outcomes may provide useful information about BV and HIV MCT.

3.0 STUDY DESIGN

This is a randomized, double blinded, controlled phase III clinical trial of antibiotics. The interventions will be conducted at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi, and in urban clinics in Lusaka, Zambia. Study participants will be randomized to receive either antibiotic treatment or placebo. Enrollment will occur at a rate of approximately 200 mother/fetal pairs per month, with approximately equal accrual (100 women per month) from QECH in Blantyre, Malawi, and four urban clinics in Lusaka, Zambia. Follow-up will continue until 12 months after the last woman delivers or until sufficient events accrue to provide adequate power for the primary endpoints.

The following two paragraphs demonstrate that there are sufficient HIV-positive women in the sites chosen to carry out the study.

In Lusaka, 36,000 deliveries occur within the health system each year. Seventy percent are seen in the second trimester, using an estimate calculated in January 1998 from a survey by Drs. Ahmed and Vermund of women delivering in Lusaka. Thirty percent are HIV infected. If the urban clinics alone were used, there are 13,000 deliveries per year, with most of the women receiving their prenatal care through the clinic's outpatient prenatal care services. Hence, in Lusaka, there are about 1100 women delivering monthly, 330 of whom are HIV infected. Therefore, the study is feasible in Lusaka since recruitment of only 100/330 HIV infected women each month will be necessary.

In Blantyre, Malawi, several cohorts of pregnant women were recruited from the QECH during the ICAR, PAVE, birth canal washing and micronutrient supplementation studies. The HIV prevalence has been well-documented; it has risen from 2% in 1985 to 30% in 1997.¹⁹ Approximately 1200 deliveries occur at QECH every month (during the HIVNET birth canal washing intervention, 6965 women gave birth at QECH during the six month duration of the study).²⁰ The investigators in Malawi have routinely monitored antenatal clinic attendance at QECH over several years. On average, 540 women attend the antenatal clinic every month (about 160 being HIV infected). Approximately 75% of the women attend hospital antenatal care in the second trimester. Therefore, about 120 women will be available for recruitment every month into the proposed clinical trial from the QECH alone, of which 100 will be enrolled. This is consistent with experience in the recruitment of the most recent cohort in 1995/96 where enrollment was initiated at 17 weeks gestation, in which a similar high proportion of women screened were enrolled.

These data provide documentation that sufficient HIV-positive subjects are present in both locations so that approximately 1000 HIV-positive women will be recruited in each location in approximately one year. Each potential participant will be provided appropriate information about the study and her informed consent will be obtained prior to enrollment.

In each site about 300 HIV-negative women will be enrolled to conceal the HIV status and avoid stigmatization of participating women. This sample of HIV-negative women will also provide valuable information on the secondary objectives (see Section 3.2). Determination of whether the use of this regimen is associated with an improvement in birthweight, a reduction in preterm birth, and a reduction in maternal chorioamnionitis in HIV-negative women, as well as whether adherence to the treatment regimen and tolerance of the antibiotics are similar in the two cohorts, are of interest. Data from the HIV-positive and negative women will not be combined in the analysis.

3.1 Selection and Enrollment of Subjects

The inclusion and exclusion criteria refer to all prospective participants (both HIV positive and negative women with the exception of their HIV status).

3.1.1 Inclusion Criteria

- HIV positive at enrollment;
- 20-24 weeks gestation;
- Resident in urban or periurban Blantyre for Malawi, or Lusaka for Zambia;
- Willing to give informed consent (for HIV testing and for enrollment into the study);
- Willing to take treatment as scheduled;
- Planning to deliver at QECH or one of the four urban study clinics in Lusaka;
- Willing to come back for follow-up visits for one year post-partum.

3.1.2 Exclusion Criteria

- Have taken antibiotics, other than treatment for syphilis or gonorrhea, within the last two weeks;
- Are allergic to penicillin, ampicillin, erythromycin, or Metronidazole;
- Have known major illnesses likely to influence pregnancy outcome including diabetes, severe renal or heart disease, or active tuberculosis, prior to randomization;
- Have known major obstetric problems such as placenta previa, ruptured membranes or multiple pregnancy prior to randomization.
- Have known central nervous system diseases, including seizures.
- Are taking anticoagulant drugs.

3.1.3 Enrollment Procedures

This study will be conducted at Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi and in four urban clinics in Lusaka, Zambia. Women will be recruited from the antenatal clinics. Informed consent for HIV counseling and testing, and screening for the study will take place. Women

in Zambia will receive their test results on the same day; women in Malawi will return to the clinic in one to two weeks to receive their test results. If the woman is eligible for the study, an enrollment visit will be scheduled at 20-24 weeks gestation; the woman will be encouraged to bring her partner to the enrollment visit. At the enrollment visit, the study will be explained to the woman and her partner (if available) and informed consent will be obtained. After informed consent is obtained, the woman will be randomized. Randomization will employ permuted block algorithms with randomized block sizes. Randomization of HIV-positive women will be stratified only by site. A separate randomization stratification will occur for HIV-negative women (see section 3.2 below).

3.2 Co-enrollment Guidelines

A sample of 300 HIV-negative women will be included in the study from each site. These women will be randomized using a separate stratified randomization scheme and followed during pregnancy and postpartum (with their babies) in a manner indistinguishable from HIV-positive mother-infant dyads. Inclusion of these HIV-negative women will allow avoidance of stigmatization associated with inclusion of only HIV-positive women from the antenatal clinics. Furthermore, with a sample of 300 women randomly selected from each site, the effect of antibiotic treatment on some of the secondary outcomes will be evaluable. For example, a study of a sample of 600 women will have more than 80% power to detect a reduction in low birth weight from 15% (the prevailing rate in Malawi and Zambia) to 10% (a reduction of one third; alpha 0.05, one-sided test). Establishing a beneficial impact of the proposed treatments among HIV-negative women may justify recommending antibiotics to all women irrespective of their HIV status. There is also potential to study the effect of the antibiotics on early infant morbidity and mortality. Therefore, generalizability of the study findings will be possible.

A sample size of 300 is based on co-enrollment of one HIV-negative woman subsequent to every three HIV-positive women in each site. One HIV-negative woman will be requested to participate following the third successfully enrolled HIV-positive woman. If the selected participant refuses or is not eligible, the HIV-negative woman immediately succeeding her will be approached.

4.0 **CLINICAL AND LABORATORY EVALUATIONS**

4.1 Pre-entry Evaluations

Appropriate pretest HIV counseling by trained HIV counselors will be provided to all women attending the antenatal clinic on the day of screening. Women who agree to be HIV tested will sign a screening informed consent form. Screening for syphilis is part of the routine antenatal care in both Malawi and Zambia. For those tested, HIV and syphilis test results will be available on the same day or within one week from the initial antenatal visit, and in general, women will be seen for a repeat

counseling visit within two weeks of testing. All women will receive post-test counseling upon return to the clinic. HIV-positive women and a sample of HIV-negative women will be requested to participate in the study; an enrollment visit will be scheduled at 20-24 weeks gestation and women will be encouraged to bring their partners.

HIV testing: Blood will be collected from all consenting women for HIV testing. In Zambia, rapid testing will be used; the Dipstick 1+2 will be used for screening and the Capillus agglutination test will be used for confirmation²¹. In Malawi, the Genetic Systems LAV EIA will be used for screening and the Wellcozyme HIV Recombinant EIA will be used for confirmation. These tests will be run on the same blood specimen to identify HIV seropositive women. HIV indeterminate results on EIA tests will be confirmed by western blot.

Syphilis testing: All women will have blood tested using RPR for screening and TPHA for confirmation. Women with a reactive syphilis test will receive treatment at no cost.

4.2 Evaluations During Pregnancy and Labor

At the enrollment visit the study procedures will be explained to the women and their partners, including the schedule of the follow-up visits. Women who agree to participate in the trial will sign a separate enrollment consent form; signature of the partner will also be requested.

Structured questionnaires will be used to collect the non-laboratory data described in the table below (Table 1). In addition, at the first antenatal visit (20-24 weeks) socio-demographic information including age, parity, maternal and paternal education, residence, socioeconomic status, marital status, and number of sexual partners will be collected on all women. The enrollment questionnaire will include symptoms of clinical AIDS (for HIV-positive women) and sexually transmitted diseases. A clinical examination form will also be completed. Table 1 summarizes these activities.

Gestational age will be determined based on patient recall of the last menstrual period, corroborated with uterine size. If uterine size is not consistent (within 2 weeks) with recall, uterine size will define the obstetric gestational age (ultrasound examinations are rarely performed at these sites; therefore estimating gestational age by this criterion will not be routinely available). In addition, at delivery, the babies will have a modified Ballard examination performed to allow for both an obstetric and pediatric estimation of gestational age. If the two are consistent within 2 weeks, the obstetric gestational age will be used; if not, the final study gestational age will be based on the Ballard examination. Staff on both locations will be trained to determine both the obstetric and pediatric gestational ages. However, this algorithm will result in a single project

gestational age which will be used whenever a project gestational age is required as part of the protocol or as an outcome.

Table 1. Non-laboratory and laboratory evaluations during pregnancy and labor.

<u>Non-Laboratory Data</u>	<u>Labs</u>
<u>Enrollment (20-24 wks):</u> Maternal Demographics Obstetric History Medical History Sexual History Gestational Age Antibiotic Usage Other Medications	BV Status (Gram Stain, , whiff test and pH) Wet mount for clue cells (Malawi only) Candida, Trichomonas (wet mount) FFN Status (Elisa) * Plasma for Zinc/Vitamin *, Viral Load [?] and CD4 Analysis [?] Vaginal Fluid For HIV Load* [?] Vaginal Fluid for Chlamydia, Gonorrhea DNA *
<u>Follow-up (26– 30 Wks)</u> Intervening Medical History Antibiotic Usage Other Medications Pill Counts/Adherence/Adverse Reactions	BV Status (Gram Stain, whiff test and pH) Wet mount for clue cells (Malawi only) Candida, Trichomonas (wet mount) FFN Status* Vaginal Fluid for HIV Load* [?] Vaginal Fluid for Chlamydia, Gonorrhea DNA*
<u>Follow-up (36 Wks)</u> Intervening Medical/OB History Antibiotic Usage Other Medications Pill Counts/ Adherence/Adverse Reactions	
<u>At Delivery</u> Length of Labor Length of ROM Clinical Chorioamnionitis Obstetric Interventions Infant Weight/Gestational Age Antibiotic Usage Pill Counts/Adherence/Adverse Reactions	Placenta, Membranes and Cord for Histology Heelstick for Infant PCR for HIV (within 24 – 48 hours) ^D Maternal Plasma for Zinc/Vitamin *, Viral Load [?] and CD4 Analysis [?] Colostrum for Viral Load* [?]

* To be stored for later analyses

[?] To be run on all HIV-positive women; however, to maintain confidentiality, specimens will be collected from all women

^D To be run on all infants of HIV-positive subjects; however, to maintain confidentiality, specimens will be collected from all infants

4.2.1 Adherence

The prenatal antibiotics, Metronidazole and erythromycin, and the peripartum antibiotics, Metronidazole and ampicillin, and placebos will be packaged in blister packs to be given to each woman at appropriate visit. The initial dose of prenatal antibiotics or placebo will be taken at the enrollment visit under direct observation, and the participant will be asked to bring the blister packs with her to her next visit at 26-30 weeks gestation. Each woman will be given the peripartum antibiotics or placebo at the 36-week visit. She will take the antibiotics or placebo at onset of labor and throughout delivery and after delivery until the course is completed. She will be asked to bring the blister packs with her to her

next visit at 46 weeks post-partum. Adherence will be assessed by the number of pills missing from the blister packs at the follow-up visits. If the blister packs are not returned, reported use will be noted. Adherence to the vitamin regimen will also be assessed through blood folate levels measured in a subset of women.

Erythromycin use is associated with various types of gastrointestinal complaints (but very few serious complications) and continued use is less than optimal for this reason. In an effort to enhance appropriate use of the drug, at randomization, each woman will be informed of the potential for mild but not harmful gastrointestinal side-effects and will be encouraged to continue using the medications. If these side effects become intolerable, rather than discontinuing, the women will be encouraged to reduce the dose, but take the medication over a longer time-period. The amount of each drug taken by the participants will be monitored.

Rarely, patients taking metronidazole and/or erythromycin have been reported to develop a peripheral neuropathy, seizures, or other CNS symptoms. For this reason, patients with central nervous system diseases, including seizures, will be excluded from participation, and any patient developing a neuropathy or other CNS sign or seizures during treatment will have the erythromycin and metronidazole or their placebos discontinued.

Since metronidazole may potentiate the action of certain anticoagulants, women taking any anticoagulant drug will also be excluded from participation.

4.2.2 Other STDs

Except for the intervention of study antibiotics (or placebo) and the addition of a vitamin/mineral supplement, the women will receive regular prenatal care. In Zambia and Malawi, women are generally screened for syphilis, and at times for gonorrhea. If reactive for syphilis or positive for gonorrhea, women are generally treated with a penicillin-like drug. Treatment of syphilis or gonorrhea with a penicillin-like drug should have little influence on the development of chorioamnionitis since the organisms involved, including *Gardnerella*, *Bacteroides*, *Mycoplasma* and *Ureaplasma*, are not generally susceptible to the penicillin-like antibiotics. For this reason, women who receive penicillin-like antibiotics or were treated for syphilis or gonorrhea within two weeks prior to randomization will not be excluded. Bacterial vaginosis is not usually screened for or treated in these settings, but if a woman complains of a symptomatic discharge, she will be appropriately treated. Candida, trichomonas and chlamydia are not routinely screened for, but occasionally are treated if symptomatic. In any case, for each woman, at

the time of enrollment, during chart review and again at delivery, the study physicians will discern which antibiotics, if any, were used during the course of pregnancy and these will be recorded. It is important to nest this clinical trial within the usual health care environment to assess its utility within the actual local medical practice. Other than the improved screening and treatment for syphilis and provision of multivitamin/mineral supplements, the usual standard of care in both Malawi and Zambia will be provided.

4.2.3 Evaluation of clinical and laboratory events

This study will investigate if BV and FFN are predictors of perinatal HIV transmission, and also if treatment of HIV-positive women with or without BV and/or FFN with the proposed antibiotic regimen results in a reduction in perinatal HIV transmission. Diagnosis of BV (or FFN positivity) will not influence the conduct of the treatment in this study. The diagnosis of BV will be determined based on a Gram stain (Nugent criteria) of vaginal fluid taken at the randomization visit. Since BV will not be used to direct therapy, the slides for Gram stain for BV will be evaluated in batch independent in time from the conduct of the trial. FFN testing is being done to answer a question related to the mechanism of HIV MCT; if MCT is more common in FFN-positive women, this would strongly suggest that chronic chorioamnionitis plays a significant role in this process.

The Gram staining method to diagnose BV requires expertise and training. Therefore, simple inexpensive methods to assess BV are desirable. Clinical criteria to diagnose BV have been developed (three of the following four criteria: vaginal pH >4.5, homogenous vaginal discharge, presence of $\geq 20\%$ clue cells, and a positive amine test), and are highly correlated with the Gram staining scoring method. In a recent study in Malawi, BV based on these clinical criteria was shown to be associated with HIV perinatal transmission¹² and increased acquisition of HIV.¹¹ Vaginal pH will be measured using pH paper on a vaginal swab obtained from lateral and posterior fornices. An amine (or whiff) test will be performed by mixing a few drops of 10% potassium hydroxide with vaginal fluid. These tests are simple and can be routinely performed in the hospital laboratories of Malawi and Zambia. In Malawi, the presence of clue cells will be evaluated by mixing vaginal fluid with a drop of normal saline on a slide and examining under high power magnification; presence of clue cells will not be evaluated in Zambia. The presence of vaginal discharge will be determined by a speculum-aided pelvic exam conducted by trained nurses. The implications for low cost screening to identify candidate women for therapy in areas of the world with less resources are obvious; hence this nested evaluation of the sensitivity, specificity and predictive value of clinical criteria to diagnose BV is an important subproject within this clinical trial.

FFN is measured using an EIA assay from vaginal fluid collected using a Dacron swab which is placed in buffer and frozen for subsequent analysis. Adeza Biochemical, Sunnyvale, CA, has provided the investigators the EIA kits necessary to determine levels of fetal fibronectin and will make kits available for this study. If FFN identifies a group at substantially increased risk for HIV MCT, this will enhance knowledge about the mechanism of MCT and it may allow future investigators the opportunity to target transmission in women at high risk for MCT.

At enrollment and approximately 4 weeks later, specimens will be collected to diagnose BV, and a wet mount preparation will be performed for detection of candida and trichomonas. A swab in buffer for FFN and a cervical swab ultimately to be used for GC, chlamydia PCR, and vaginal viral load evaluations will be collected. Analysis of the Gram stains will be conducted for all women. FFN and bacterial DNA (chlamydia and gonorrhea) samples will be stored to be run in batch when feasible.

Blood samples will be collected from each woman at enrollment. The blood from HIV-positive women only will be tested for CD4 counts and viral load. The blood from each woman will be tested in the future for nutrient analyses to determine adherence with taking the vitamin/mineral supplement and for potential future studies relating MCT to nutritional status.

4.3 Evaluations at Delivery

At delivery, clinical chorioamnionitis will be defined by the presence of a temperature $>100^{\circ}\text{F}$ during labor in association with any of the following: 1) fetal tachycardia >160 BPM, 2) significant abdominal tenderness, 3) a purulent cervical discharge, or 4) a foul smelling infant or placenta.

After delivery, the placenta will be brought to the laboratory in a sealed plastic bucket containing 500 ml 10% formol saline and weighed. The membranes will be rolled in a standard fashion, and after fixation, two sections at least 2 cm apart will be evaluated. Two sections of the cord, one close to the placenta and the other near the fetus will be obtained, as will two representative sections of the placenta. The specimens will be fixed in 10% neutral buffered formalin, processed for routine paraffin embedding, embedded, cut at 5 μ micron thickness and stained by routine hematoxylin-eosin method.

Slides will be examined without knowledge of outcome or maternal HIV status by on-site pathologists (Dr. Liomba in Malawi and Dr. Mudenda in Zambia). To promote consistency between sites, the two on-site pathologists will undergo joint training to standardize diagnosis. Also, after reading the slides, ten percent of the slides from each site will be exchanged between sites to be reexamined by the other pathologist. If there are discrepancies in diagnosing chorioamnionitis between sites,

an outside expert will be retained to reexamine a portion of the slides from each site for quality control.

Histologic chorioamnionitis will be based on qualitative assessment of the membranes, cord and placenta basalis, looking for mononuclear and polymorphonuclear white cells using a standard protocol. A grading system which divides the histologic chorioamnion evaluation into normal and mild, moderate and severe chorioamnionitis will be used. It will be noted if the predominant infiltrate is mononuclear or polymorphonuclear so that the chorioamnionitis may be divided into chronic or acute. The presence of findings suggestive of placental malarial infiltration will also be noted.^{22,23,24} (See Appendix VI for evaluation form.)

Following delivery, mothers and infants will be evaluated for evidence of disease and adverse reactions to medications. Blood specimens will be collected from each woman and will be stored for future micronutrient analysis. A portion of the blood collected from HIV-positive women will be tested for viral load and CD4 counts. Colostrum and breast milk will be collected from each woman; specimens from HIV-positive women will be frozen for later analysis, while specimens from HIV-negative women will be discarded. Placental tissue will be stored so that examination of the placenta by in situ and immunohistochemistry using a novel and highly sensitive method utilizing tyramide-fluorophors may be conducted. This method can simultaneously detect HIV RNA, HIV DNA, HIV proteins and cellular proteins and help delineate the role of chorioamnionitis and HIV expression. A section of the placenta will also be kept to examine for malaria parasites and histologic changes associated with malaria. Malaria is endemic in Malawi and Zambia and is a known risk factor for preterm birth and low birthweight.²⁵

The investigators plan to obtain biologic specimens which have the potential to answer questions related to mechanisms of transmission, but are not crucial to answer the primary question about efficacy of antibiotics for reducing HIV MCT. These include the testing for BV; FFN; viral load in blood, vagina and breast milk; evidence of malaria in the placenta; blood for vitamin/mineral measurements, etc. If, during the pilot phase, it appears that obtaining any of these specimens will reduce the overall ability to carry out this study, the collection of these specimens will be deleted from the protocol.

4.4 Post-Delivery Evaluations (Table 2)

At 24-48 hours and at 4-6 weeks of age all infants will have a heelstick. Specimens from infants of HIV-positive women will be tested for HIV RNA using PCR; specimens from infants of HIV-negative women will be discarded. Infants who are positive using the definition of an infected infant (7.51) will have reached the primary endpoint. Because a second primary endpoint includes being alive and free of disease at 1 year of age, infants of HIV-positive women who did not test positive for HIV RNA at 24-48 hours and 4-6 weeks will be tested for

HIV antibody at 9 months and 12 months, and all who are negative will be considered free of HIV; to maintain confidentiality, all other infants will also have a heelstick, but the specimens will be discarded prior to testing. Antibody positive infants will have PCR for HIV RNA performed and those positive using definitions in 7.51 will be considered positive for this endpoint. If the recommendations are still current at the time study infants are born, each infant diagnosed as HIV-positive will be provided with prophylactic Bactrim or an equivalent medication during the course of the study.

Following delivery (24-48 hours), at 4-6 weeks, 3 months, 6 months, 9 months and one year of age, questions related to infant health, maternal health and breastfeeding practices will be asked. The interval visits at 3 and 6 months are justified in order to determine if there is interval morbidity and mortality and to keep the cohort intact until the one-year endpoint. At each post-delivery visit, a clinical assessment form will be completed for the mother and the newborn. These activities are summarized in Table 2.

Because there is concern that some antibiotic treatment may lead to an increase in neonatal sepsis with more virulent organisms, occurrence of neonatal sepsis prior to discharge from the hospital or at the 4-6 weeks evaluation will be monitored. The DSMB will evaluate mortality and severe morbidity in the treatment and placebo groups at 3 month intervals to ensure that the treatment is not causing harm.

Breast feeding is nearly universal in both sites. The investigators are aware of the risk associated with HIV transmission to the baby if breastfeeding is prolonged (for example, beyond the critical 6 months of age). Currently, breastfeeding is encouraged in Malawi and Zambia during the entire infancy since alternative safe measures are not generally available. However, women will be adequately counseled and the risks and benefits of breastfeeding will be explained thoroughly at each visit to help women make an informed decision. During the entire study period the research team will be guided by the advice and recommendations adopted in each country.

It is understood that some infants will likely become HIV infected due to breast feeding prior to evaluation at 4-6 weeks. There is no method available to allow distinction between infection occurring during the perinatal/peripartum period and secondary infection due to early breast feeding.

Table 2. Non-laboratory and laboratory evaluations during follow -up.

Non-Laboratory Data	Labs
<p>At 24-48 hours; 4 – 6 wks; 3, 6, and 9 month 1 year Infant Viability and Health, Maternal Health Breast vs. Formula Feeding</p>	<p>24-48 hours, 4-6 weeks Heelstick for Infant PCR for HIV^D</p> <p>9 months, 1 year Antibody testing^D Heelstick for Infant PCR for HIV (at 1 year only if status not clear)^D</p> <p>4-6 weeks; 3, 6, and 9 months; 1 year Breast Milk collection*</p>

* Specimens from HIV-positive women will be stored for later analysis of viral load; specimens from HIV-negative women will be discarded.

^D To be run on infants of HIV-positive women only whose HIV status is undetermined; specimens collected from infants of HIV-negative women and infants who have been determined to be HIV-positive at 4-6 week PCR will be discarded.

5.0 DATA COLLECTION AND MONITORING AND ADVERSE EXPERIENCE REPORTING

5.1 Records to Be Kept

Case Report Forms (CRF) will be provided for each subject. Subjects will not be identified by name on any study documents. Subjects will be identified by an identification number unique for each participant; this will be provided by the HIVNET Statistical Center upon randomization. At screening, all women will be provided a temporary screening ID to track clinical and laboratory results. The use of the screening ID will be discontinued upon randomization.

All data on the CRF must be legibly recorded in black ink. A correction should be made by striking through the incorrect entry with a single line and entering the correct information adjacent to it. The correction must be initialed and dated by the investigator or a designated, qualified individual.

5.2 Data Management and Documentation Guidelines

Instructions concerning the recording of study data on case report forms, or the entry of data in the computerized database, will be provided by the HIVNET Statistical Center.

The HIVNET Statistical Center will coordinate data management for the trial.

5.3 Regional Monitoring

5.3.1 Site visits by FHI study monitors will be made in accordance with HIVNET policy to monitor the quality of data collected in the research

records, the accuracy of the data entered in the database, and compliance with all regulatory requirements associated with clinical trials.

5.3.2 Site visits will be made at the Zambia and Malawi HIVNET sites at regular intervals.

5.3.3 The investigator will make study documents (e.g., consent forms, drug distribution forms, case report forms) and pertinent hospital or clinic records readily available for inspection by the FHI site monitor and pertinent NIH staff for confirmation of the study data.

5.4 Adverse Experience Reporting

An adverse event is defined as any health related reaction, effect, toxicity or abnormal laboratory result that a patient experiences during the course of a study irrespective of relationship to study treatment.

Adverse experience should be reported according to reporting requirements as described in Appendix III.

6.0 STUDY TREATMENT

6.1 Drug Regimens, Administration and Duration

At 20 – 24 weeks, the women randomized to the treatment arm will receive Metronidazole 250 mg TID and erythromycin 250 mg TID for 7 days. Women randomized to the control group will receive identically appearing placebos. All women in both treatment and control arms will receive a standard vitamin/mineral preparation daily from enrollment in the study until delivery. This preparation will include 30 mg iron, 400 mcg folic acid, 5000 IU Vitamin A, 400 IU Vitamin D, 30 IU Vitamin E, 50 mg Vitamin C, 2 mg Vitamin B₁, 3 mg Vitamin B₂, 3 mg Vitamin B₆, 5 mcg Vitamin B₁₂, 20 mg Niacin, 250 mg Calcium, 150 mcg Iodine, 100 mg Magnesium, and 15 mg Zinc.

With the onset of labor and/or rupture of membranes, rapid antibiotic entrance into the amniotic fluid is important. Metronidazole 250 mg, in addition to ampicillin 500 mg will be administered orally every 4 hours to women randomized to the treatment arm to prevent the acute chorioamnionitis associated with membrane rupture. Both of these drugs achieve satisfactory serum and amniotic fluid levels within 1-2 hours after oral administration. Since the secondary outcomes include maternal infection and mortality, each woman will be asked to continue using the medications after delivery, three times per day, until the course is completed. Women randomized to the control group will receive identically appearing placebos.

6.2 Drug Formulation

Metronidazole and erythromycin will be purchased from Anda Generics, Fort Lauderdale, FL. The placebos for the metronidazole and erythromycin will be manufactured and the metronidazole and erythromycin will be re-encapsulated by Clinical Encapsulation Services, Cobleskill, NY. The placebos for the ampicillin will be manufactured and the ampicillin will be procured and re-encapsulated by ProClinical Laboratories, Philadelphia, PA.

6.3 Drug Supply, Distribution and Pharmacy

The Metronidazole and erythromycin distributed at the enrollment visit and the Metronidazole and ampicillin distributed at the 36-week visit will be packaged in blister packs. Study participants will be asked to return the blister packs at their next visit so that adherence may be evaluated.

The protocol pharmacist is required to maintain complete records of all study agents received from the drug companies and subsequently dispensed.

6.4 Concomitant Medications

Any concomitant medication, if deemed medically necessary, will be permitted for either the mother or neonate while on-study; the administration of concomitant medication will be recorded on CRFs. Because of the reported interaction between metronidazole and alcohol, all women in the study will be asked to refrain from alcohol use during the trial, as reflected in the informed consent form.

6.5 Toxicity Management

Allergic reactions are possible with any drug. Severe anaphylactic types of reactions have been reported with penicillin use (1/50,000). Gastrointestinal side effects including nausea and vomiting and diarrhea have been reported with erythromycin use. If the toxicity appears to be a significant allergic reaction, both drugs will be stopped immediately. However, if the problem appears to be mild gastrointestinal discomfort associated with erythromycin use, the Metronidazole will be continued at full dose. If necessary, the daily erythromycin dose may be reduced, but will not be discontinued unless the woman clearly cannot tolerate the medication. If there is nausea and vomiting or diarrhea, the women will be asked to decrease only the erythromycin, first to 2 tablets per day, and then, if necessary, to one per day until the symptoms abate. If a participant experiences wheezing or rash compatible with study drug reaction while on study drugs, the study drugs will be stopped.

6.6 Criteria for Treatment Discontinuation

Study participants may be discontinued from treatment for the following reasons:

- The subject refuses further treatment and/or follow-up evaluations.
- The investigator determines that further participation would be detrimental to the subject's health or well-being.

- The subject experiences a severe allergic reaction to the study drugs.

6.7 Other Infections

A number of questions have arisen about the use of other treatments, especially the use of antibacterial agents that may obscure the results of this study. No appropriate treatment will be withheld because a woman is enrolled in this study. Therefore, if a woman has a symptomatic vaginal infection that would normally be treated in Zambia and Malawi, appropriate treatment will be given. If it is standard treatment to use antibiotics in the face of ruptured membranes for more than 24 hours, or with maternal fever, they will be utilized. If newborns are normally treated with antibiotics following maternal chorioamnionitis they will be so treated. The individual treatments received by study subjects and the prevailing clinical practices that may influence MCT will be documented, but no attempt will be made to restrict what is considered routine medical care.

If during study drug administration a woman has a condition requiring antibiotic use, such as a urinary tract infection, chorioamnionitis or ruptured membranes of more than 24 hours, the woman will be treated with routine antibiotics in addition to the study drugs. Because the active arm of the study uses a relatively low dose of antibiotics, additional use of antibiotics should not place the woman at increased risk. The alternative option, unblinding the woman and providing antibiotics if she is on the placebo, would result in significant risk to bias the study. However, if the physician, because of severe allergic reaction or any other cause, feels it is necessary or in the patient's best interest to unblind the study products, the nature of the study drugs will be made available to the attending physician. The list will be kept by the in-country pharmacist, who will be the only person able to break the code.

7.0 STATISTICAL CONSIDERATIONS

7.1 General Design Issues

This is a randomized, double blinded, controlled Phase III clinical trial of antibiotics to reduce chorioamnionitis-associated perinatal HIV transmission. The interventions will be administered to pregnant women during the second and third trimesters of pregnancy, in order to interrupt vertical transmission of HIV.

7.2 Endpoints

7.2.1 Primary endpoints

- Infant HIV infection as determined by two separate positive RNA PCR on heel stick dried blood spots on filter paper at 4-6 weeks of age.²⁶
- Composite of infant HIV infection and mortality at one year of age.

7.2.2 Secondary endpoints

- Gestational age and weight at birth
- Proportion of infants born preterm (<37 weeks) or with low birthweight (<2500g)
- Rates of clinical and histologic chorioamnionitis
- Rates of neonatal/infant morbidity and mortality
- Rates of maternal morbidity and mortality
- Safety/tolerance of antibiotic treatment given to pregnant women from Malawi and Zambia

7.2.3 Definition of gestational age is found in section 4.2. Preterm birth will be defined as <37 weeks, but evaluation of preterm birth at earlier gestational ages defined as <35 and <32 weeks will also be done. The definition of histologic chorioamnionitis is found in section 4.2.3. Infant mortality will be divided into neonatal, defined as <28 days, and post-neonatal, defined as 28 to 365 days. Evaluation of neonatal morbidity will include suspected or documented sepsis (based on physician examination or positive culture), pneumonia (based on physician examination or xray), and suspected or documented respiratory distress syndrome (based on examination or x-ray). The length of stay on initial hospitalization will be documented. Seizures and the presence of signs and symptoms of meningitis will be noted. Post-discharge medical problems such as seizures, pneumonia, meningitis, severe diarrhea, and all hospital readmissions and diagnoses will be noted. At each visit the infant will be weighed and the height and head circumference measured so that deviations from normal growth (<10th percentile for an African standard) may be determined. Medications used, such as any antibiotic treatment will be recorded.

Data on the mothers' health during the initial hospitalization and following discharge will also be collected. Length of hospital stay and readmissions will be noted as will the use of any antibiotics for treatment of infections. Maternal diagnoses including chorioamnionitis, post-partum endometritis, wound infections, urinary tract infections, pneumonias, etc., will be recorded. Any adverse reactions to the medications as well as the adherence to the medications provided will also be tracked.

7.3 Randomization and Blinding Procedures

Participants will be randomized at 20 - 24 weeks gestation in a double-blinded fashion to receive either the active agent (Metronidazole/erythromycin/ampicillin) or matched placebo. The randomization will be designed by the HIVNET Statistical Center and employ permuted block algorithms with varying block size, blocked within study site to ensure that balance between assignments is maintained within each study site. Study drug will be packaged according to the randomization and sent to the study site. Randomization will be performed on

site by assigning study drug to participants in sequential order. These procedures will be coordinated with the HIVNET Statistical Center and detailed in the Manual of Operations. HIV-positive and HIV-negative subjects will be randomized in separate strata.

7.4 Sample Size and Accrual

The rate of HIV-1 transmission is a primary endpoint for this study. The control arm 4-6 weeks transmission probability is estimated to be 25% in both Malawi and Zambia. This estimate is based on testing more than 1000 babies at 6 and 12 weeks in the birth canal washing study in Malawi using DNA PCR,²⁷ and pilot studies with similar results in Zambia. For a one-sided 0.025-level chi-square statistic to provide 90% power to detect a 25% reduction in vertical transmission, 1828 mother/infant pairs would be needed in the trial. However, allowing for up to 3% loss-to-follow-up rates during pregnancy, and 10% missing information post-delivery, the majority of this due to early mortality,²⁸ the trial will require 2100 mother/infant pairs. The investigators will work very hard to achieve the proposed 3% loss to follow-up. However, if that rate is not achieved, sample sizes will be recalculated so that they reflect the actual lost-to-follow-up rates. A 3% lost-to-follow-up rate was achieved in Malawi in the chlorhexidine study²⁷.

Because AZT is not routinely available in either country, the current sample size calculations described above are based on the assumption that prenatal AZT will not be provided to trial participants. In the event that AZT treatment becomes available, the sample size will be recalculated to reflect an approximate 50% reduction in the expected rate of transmission.

7.5 Monitoring and Analysis

An intent-to-treat analysis will be conducted using the entire sample from both sites. The incidence of HIV-1 transmission and mortality rates will be determined among infants in each study arm. Binary endpoints will be compared between treatment arms using contingency tables or, if time dependent (death/drop-out), Kaplan Meier curves. Adjustment for potential confounders will be done through logistic regression or proportional hazards models. Single continuous outcomes (viral load, CD4 counts, etc.) will be compared with t-tests or non-parametric analysis. If needed, variable transformations such as logarithms will be used. Adjustment for confounders will be made with linear models. Repeated measures such as symptoms and anthropometric indices will be evaluated using generalized estimation equations.

An initial safety analysis will be performed after the first three months of accrual. Formal interim analyses of efficacy will then be performed at approximately six month intervals during the projected 18-24 month study duration. Recommendations for early termination of positive or negative results will be guided by the symmetric group sequential O'Brien-Fleming Boundary. The O'Brien-Fleming design allows for early termination if extreme initial results are seen, while essentially allowing employment of the standard single stage one-sided 0.025-level test statistic at the

final analysis if it is reached. This enables one to maintain the power of the single-stage design in the presence of interim monitoring without having to increase the maximum sample size. The Lan-Demets implementation of the O'Brien-Fleming use function will be employed to define proper significance levels at the time of each formal interim analysis by the DSMB.

Timing of transmission will be estimated by assuming that if the infant HIV PCR test was positive within 24 to 48 hours of birth, that transmission likely occurred prenatally, while if it was negative within 24 to 48 hours of birth but positive at 4-6 weeks, that MCT occurred in the intrapartum period. The investigators are aware that some of the infants who turn HIV-positive between discharge and 4-6 weeks will do so because of breast feeding, but at this time have no way to determine the proportion of the converters due to breast feeding vs. perinatal transmission. Infant tests which turn positive at 6 and 12 months of age will be considered to be related to late breastfeeding transmission.

In addition to viral load and CD4 counts, several variables will be evaluated for their relationship to MCT in each group including BV and FFN status, plasma serum zinc and vitamin A levels, the presence of STDs, histologic chorioamnionitis, length of PROM and labor, obstetric interventions, and gestational age and/or preterm birth.

7.5.1 Definition of HIV Infected/Uninfected Infant for HIVNET Perinatal Protocols

Following is the standard HIVNET definition adopted by the HIVNET Perinatal Working Group; if this definition changes before implementation of this protocol, adoption of these alterations will be considered so that a standard definition is used for all HIVNET perinatal transmission protocols:

An infant less than 15 months of age will be considered to be infected with HIV if two separate peripheral blood specimens from different days are drawn and each specimen is positive by at least one of the following assays: HIV-1 culture, HIV-1 DNA PCR, HIV RNA RT-PCR. At least one of these tests will be done in a laboratory which is approved to perform the assay for protocol testing. A positive result will be confirmed no later than the next scheduled visit and sooner if possible.

If PCR is used, then the Roche HIV-1 DNA AMPLICOR or the qualitative Roche HIV-1 RNA AMPLICOR assay will be used with a primer pair capable of efficiently amplifying and detecting all major HIV-1 group M subtypes.

Infants greater than 15 months of age who are reactive for HIV-1 antibody by two different EIAs or HIV-1 Western Blot will be considered to be HIV-1 infected.

A non-breastfed infant born to an HIV-infected mother will be considered to be uninfected with HIV when two separate peripheral blood specimens are drawn on different days and both are negative either for HIV DNA, HIV RNA, and/or HIV culture. All of these tests will be performed in a laboratory which is approved to perform the assay for protocol testing. Specimens will be drawn at least 4 weeks apart and will be drawn when the infant is four weeks of age or older and has been off antiretrovirals for at least two weeks. At least one specimen will be drawn when the infant is greater than 8 weeks of age. Infants who are >9 months of age will be considered to be uninfected if they are nonreactive by EIA for HIV-1 antibody.

A breastfed infant born to an HIV-infected mother will be considered to be HIV uninfected if after 8 weeks from the time of weaning two separate peripheral blood specimens are drawn on different days and both are negative either for HIV DNA, HIV RNA and/or HIV culture. All of these tests will be performed in a laboratory which is approved to perform the assay for protocol testing. Specimens will be drawn at least 4 weeks apart and after the infant has been off antiretrovirals for at least two weeks.

In cases where there is one positive specimen for HIV DNA, HIV RNA, or HIV culture, the infant will be considered uninfected only if the last two subsequent specimens are negative by the same initially positive marker and one other marker.

8.0 HUMAN SUBJECTS

8.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent documents (Appendix IV) and any subsequent modifications will be reviewed and approved by the Institutional Review Boards or Ethics Committees responsible for oversight of the study. The mother must give written informed consent for herself and her baby's participation in the study. According to Federal regulations, for studies in fetuses *in utero* when the purpose of the study is to meet the health needs of the fetus, and the fetus will be placed at minimum risk necessary to meet these needs, or the risk to the fetus is minimal and the purpose of the study is to gain important biomedical knowledge that cannot be obtained by other means, the father's written informed consent is also required, unless his identity or whereabouts cannot reasonably be determined, or he is not reasonably available, or the pregnancy resulted from rape. The informed consents will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the mother (and father, if applicable).

8.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified only by a coded number to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done only with coded numbers. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the HIVNET or the NIAID.

8.3 Study Discontinuation

With proper justification, the study may be discontinued at any time by the HIVNET Scientific Steering Committee or the NIAID.

9.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by DAIDS and HIVNET policies. Any presentation, abstract, or manuscript will be made available for review by the HIVNET Manuscript and Presentation Review Committee prior to submission.

10.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention.

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APPENDICES

- I. SCHEDULE OF EVALUATIONS**
- II. DIVISION OF AIDS TOXICITY TABLE FOR GRADING SEVERITY OF PEDIATRIC (< 3 MONTHS OF AGE) ADVERSE EXPERIENCES
and
DIVISION OF AIDS TOXICITY TABLE FOR GRADING SEVERITY OF PEDIATRIC (\geq 3 MONTHS OF AGE) ADVERSE EXPERIENCES**
- III. ADVERSE EXPERIENCE REPORTING GUIDELINES**
- IV. SAMPLE INFORMED CONSENT**
- V. “CHORIODECIDUAL INFLAMMATION: A POTENTIALLY PREVENTABLE CAUSE OF PERINATAL HIV TRANSMISSION?”**
- VI. EVALUATION OF PLACENTA**
- VII. TIMELINE**

APPENDIX I SCHEDULE OF EVALUATIONS

Maternal Evaluations	Pre-entry/ Screening	Enrollment (20-24 weeks)	26-30 weeks	36 weeks	Labor and Delivery	Infant Follow-up visits (4-6 weeks; 3, 6, 9 12 months)
Laboratory:						
HIV test (using two different EIA or rapid tests)	X					
Syphilis test (RPR for screening, TPHA for confirmation)	X					
BV status [Gram Stain, clue cells (Malawi only), whiff test, and pH]		X	X			
Presence of Candida, Trichomonas		X	X			
FFN status (ELISA) ¹		X	X			
Plasma for: Zinc/Vitamin ¹ , Viral Load and CD4 analysis ²		X			X	
Vaginal Fluid for: HIV load ^{1,2} , other STD DNA (chlamydia and gonorrhea) ¹		X	X			
Placenta, membranes and cord for histology					X	
Colostrum for Viral Load ³					X	
Breast Milk for Viral Load ³						X
Non-Laboratory						
Informed consent	X	X				
Randomization		X				
Demographics		X				
Obstetric/Medical History		X	X	X		
Sexual History		X				
Gestational Age		X			X	
Antibiotic/other medication usage		X	X	X	X	
Pill counts/adherence			X			X (4-6 weeks only)
Adverse events			X	X	X	X (Serious only after 4-6 weeks)
Length of labor/ROM					X	
Clinical chorioamnionitis					X	
Obstetric interventions					X	
General Health						X
Breastfeeding practices						X

¹ Specimens to be stored for later analysis

² In HIV-positive women only

³ Specimens will be collected from all participants; however, only the specimens from HIV-positive women will be tested.

Neonate Evaluations

Neonate Evaluations	Birth (24-48 hours)	4-6 weeks	3 months	6 months	9 months	12 months
Laboratory:						
HIV RNA PCR ⁴	X	X			X ⁵	X ⁵
HIV Antibody ⁴					X	X
Non-Laboratory:						
Weight, Height, Head Circumference	X	X	X	X	X	X
Gestational Age	X					
General Health	X	X	X	X	X	X

⁴ Blood specimens will be collected from all infants; however, only the specimens from infants born to HIV positive women who have not yet been classified as HIV infected according to the definition in section 7.51 will be tested.

⁵ Infants who test antibody positive at 9 or 12 months will have PCR for HIV RNA to confirm infection status.

**APPENDIX II. DIVISION OF AIDS TOXICITY TABLE FOR GRADING SEVERITY OF
PEDIATRIC (< 3 MONTHS OF AGE) ADVERSE EXPERIENCE (November, 1993)**

All values here are for term newborns. Preterm infants should be judged by a comparison of local normal ranges and the newborn ranges identified here.

PARAMETER	GRADE 1	GRADE 2	GRADE 3	GRADE 4
HEMATOLOGY				
Hemoglobin				
1-7 days old	13.0-14.0	12.0-12.9	<12	Cardiac Failure 2ndary to Anemia
8-21 days old	12.0-13.0	10.0-11.9	<10.0	Cardiac Failure 2ndary to Anemia
22-35 days old	9.5-10.5	8.0-9.4	<8.0	Cardiac Failure 2ndary to Anemia
36-56 days old	8.5-9.4	7.0-8.4	<7.0	Cardiac Failure 2ndary to Anemia
57-90 days old	9.0-9.9	7.0-8.9	<7.0	Cardiac Failure 2ndary to Anemia
Abs Neutrophil Ct				
1 day old	5000-7000	3000-4999	1500-2999	<1500
2-7 days old	1750-2500	1250-1749	750-1249	<750
8-56 days old	1200-1800	900-1199	500-899	<500
57-90 days old	750-1200	400-749	250-399	<250
Bilirubin				
<7 days old		20-25	26-30	>30
7-60 days old	1.1-1.9xN	2.0-2.9xN	3.0-7.5xN	>7.5xN
61-90 days old	1.1-1.9xN	2.0-2.9xN	3.0-7.5xN	>7.5xN
Creatinine				
<7 days old	1.0-1.7	1.8-2.4	2.5-3.0	>3.0
7-60 days old	0.5-0.9	1.0-1.4	1.5-2.0	>2.0
61-90 days old	0.6-0.8	0.9-1.1	1.2-1.5	>1.5

Cr Clearance				
<7 days old	35-40	30-34	25-29	<25
7-60 days old	45-50	40-44	35-39	<35
61-90 days old	60-75	50-59	35-49	<35
Low Calcium				
<7 days old	6.5-6.9	6.0-6.4	5.5-5.9	<5.5
7-60 days old	7.6-8.0	7.0-7.5	6.0-6.9	<6.0
61-90 days old	7.8-8.4	7.0-7.7	6.0-6.9	<6.0
High Calcium				
<7 days old	12.0-12.4	12.5-12.9	13.0-13.5	>13.5
7-60 days old	10.5-11.2	11.3-11.9	12.0-13.0	>13.0
61-90 days old	10.5-11.2	11.3-11.9	12.0-12.9	>= 13.0

DIVISION OF AIDS TOXICITY TABLE FOR GRADING SEVERITY OF PEDIATRIC (≥ 3 MONTHS OF AGE) ADVERSE EXPERIENCES (revised: April, 1994)

PARAMETER	GRADE 1	GRADE 2	GRADE 3	GRADE 4
HEMATOLOGY				
Hemoglobin > 3 mo. - < 2 y.o.	9.0-9.9	7.0-8.9	<7.0	Cardiac Failure 2ndary to anemia
Hemoglobin \geq 2 y.o.	10-10.9	7.0-9.9	<7.0	Cardiac Failure 2ndary to anemia
Abs Neutrophil Ct	750-1200	400-749	250-399	<250
Platelets		50,000-75,000	25,000-49,999	<25,000 or bleeding
PT	1.1-1.25xN	1.26-1.5xN	1.51-3.0xN	>3xN
PTT	1.1-1.66xN	1.67-2.33xN	2.34-3.0xN	>3xN
GASTROINTESTINAL				
Bilirubin	1.1-1.9xN	2.0-2.9xN	3.0-7.5xN	>7.5xN
AST (SGOT)	1.1-4.9xN	5.0-9.9xN	10.0-15.0xN	>15.0xN
ALT (SGPT)	1.1-4.9xN	5.0-9.9xN	10.0-15.0xN	>15.0xN
GGT	1.1-4.9xN	5.0-9.9xN	10.0-15.0xN	>15.0xN
Pancreatic Amylase	1.1-1.4xN	1.5-1.9xN	2.0-3.0xN	>3.0xN
Total Amylase + Lipase*	1.1-1.4xN	1.5-2.4xN	2.5-5.0xN	>5.0xN
Uric Acid	7.5-9.9	10-12.4	12.5-15.0	>15.0 or Gout
CPK	See Neuromuscular Toxicity			
Abdominal Pain	Mild	Moderate- No Rx Needed	Moderate- Rx Needed	Severe- Hospital and Rx
Diarrhea	Soft stools	Liquid stools	Liquid Stools & Mild Dehydration Bloody stools	Dehydration requiring IV therapy or Hypotensive Shock
Constipation	Mild	Moderate	Severe	Distention and Vomiting
Nausea	Mild	Moderate- Decreased po intake	Severe-Little po intake	Unable to ingest food or fluid for >24 hours
Vomiting	<1 episode/day	1-3 episodes/day or duration >3d	>3 episodes/day or duration >7d	Intractable Vomiting

Comments:

* Both amylase and lipase must be elevated to the same grade or higher (i.e. if total amylase is Grade 4, but lipase is only Grade 1, the Toxicity Grade is 1. In pediatric HIV patients, the most common source of serum amylase is the salivary glands. Salivary amylase elevations are generally not clinically significant. When amylase is released from damaged pancreatic cells, it can be a marker of pancreatitis. In most cases of clinical pancreatitis, lipase will also be elevated. However, lipase is also a non-specific marker. Combined elevation of amylase and lipase (each >5 x normal) often indicates pancreatic disease and requires evaluation. However, in the absence of pancreatic disease, drug can be resumed even at Grade 3 and 4 toxicities.

APPENDIX II (Cont.)

PARAMETER	GRADE 1	GRADE 2	GRADE 3	GRADE 4
RENAL AND ELECTROLYTES				
CREATININE				
2 Month-2 Years	0.6-0.8	0.9-1.1	1.2-1.5	>1.5
2 Years-Adolescent	0.7-1.0	1.1-1.6	1.7-2.0	>2.0
Adolescents	1.0-1.7	1.8-2.4	2.5-3.5	>3.5
Creatinine Clearance	60-75 cc/min/1.73 m ²	50-59 cc/min/1.73 m ²	35-49 cc/min/1.73 m ²	<35 cc/min/1.73 m ²
ELECTROLYTES				
High Sodium	145-149		150-155	>155 or mental status changes
Low Sodium	130-135		129-124	<124 or mental status changes
High Potassium	5.0-5.9	6.0-6.4	6.5-7.0	>7.0 or Cardiac arrhythmias
Low Potassium	3.0-3.5	2.5-2.9	2.0-2.4	<2.0
High Calcium	10.5-11.2	11.3-11.9	12.0-12.9	>=13.0
Low Calcium	7.8-8.4	7.0-7.7	6.0-6.9	<6.0
Low Magnesium	1.2-1.4	0.9-1.1	0.6-0.8	<0.6 or Cardiac arrhythmias
Hypoglycemia	55-65	40-54	30-39	<30 or Mental status changes
Hyperglycemia	116-159	160-249	250-400	>400 or Ketoacidosis
Proteinuria	Tr-1+ <150 mg/day	2+ 150-499 mg/day	3+ 500-1000 mg/day	4+, or nephrotic syndrome >1000 mg/day
Hematuria	Microscopic <25 cells/hpf	Microscopic >=25 cells/hpf	Gross	Obstruction or Transfusion requirement
<p>Comments Calcium values are corrected for albumin concentration. CrCl values do not apply to infants <2 months old.</p>				
OTHER				
Allergy	Pruritis without Rash	Pruritic Rash	Mild Urticaria	Severe Urticaria Anaphylaxis, Angioedema
Drug Fever (Rectal)		38.5-40	>40	Sustained Fever: >40, >5 days
Cutaneous		Diffuse maculo-papular rash, dry desquamation	Vesiculation, ulcers	Exfoliative dermatitis, Stevens-Johnson or Erythema multiforme, Moist desquamation
Stomatitis	Mild discomfort	Painful, difficulty swallowing, but able to eat and drink	Painful: unable to swallow solids	Painful: requires IV fluids

APPENDIX II (Cont.)

SYMPTOM	GRADE 1	GRADE 2	GRADE 3	GRADE 4
CENTRAL NERVOUS SYSTEM				
Seizures	None	1 Uncomplicated Sz +/- Temp Elevation	1 Sz/Month for >=2 Consecutive Months Or 3 Sz over 6 Months; No Temp Elevation	>1 Sz/Month; No Temp Elevation; No Decrease in Sz Frequency Despite dose reduction
Seizures are a ubiquitous symptom of numerous systemic or CNS disturbances; alternative explanations should be vigorously sought and eliminated. Status epilepticus represents a severe end of the seizure spectrum, but should be considered as a single seizure event. The need for chronic or acute anticonvulsant medication should be made on a clinical basis. Seizures as a manifestation of drug toxicity are usually primarily generalized. Focal (partial onset) seizures are suggestive of focal central nervous system pathology and should be appropriately investigated, although they may be a manifestation of drug toxicity. Beware of focal seizures which secondarily generalize; these should be approached diagnostically as partial onset seizures. Children with underlying epileptic conditions who experience persistent breakthrough seizures despite maximal anticonvulsant therapy coincident with beginning the trial medication should be considered Grade 4.				
Headache	<=1/Month <2 Hrs duration Mild	>1/Month >2 Hrs Duration Moderate to Severe Responds to non-narcotic analgesia or prophylaxis	>2/Month >2 Hrs Duration Moderate to Severe Responds to narcotic analgesia, or does not respond to prophylaxis	>4/Month; >2 Hrs Duration; Moderate to Severe; Non-Responsive to narcotic Analgesia; or persistently Recurrent despite prophylaxis No decrease in frequency or Severity despite dose reduction
Headache is a non-specific symptom, but may be a symptom of CNS/intracranial pathology. Appropriate diagnostic measures should be pursued. Duration refers to the waxing and peak phases, not to the resolution/waning phases of the headache. Mild refers to a grade of headache pain which does not affect function or activity. Moderate to severe refers to a grade of headache which affects function or activity.				
Mental Status And Behavior	Changes which do not Affect Function	Changes requiring pharmacologic or other therapy; or mild lethargy, sedation or somnia which resolves with rest	Changes not improved by drugs or other therapies; or onset of confusion, memory impairment, lethargy, sedation, or somnia which does not respond to rest	Onset of delirium, obtundation, coma, or psychosis, or Grade 3 toxicity which does not respond to dose reduction
Behavior refers to the development of attention deficits with or without hyperactivity, depression, mania, agitation, sleep disorders, phobias, obsessive-compulsive behaviors, or anxiety. Mental status refers to the level of consciousness, memory function, language and analytical operations, and non-dominant hemisphere functioning. Alternative explanations should be sought.				
Balance & Posture	None	None	Ataxia, dizziness, vertigo, tremor, impaired postural balance	Onset of movement disorder; or Grade 3 toxicity which does not respond to dosage adjustment
"Ataxia" can be mistakenly diagnosed in the face of central weakness or peripheral neuropathy, which should not be considered a drug toxicity of this category. Movement disorders refer to tardive or other dyskinesias, dystonias, chorea, or ballismus. Alternative explanations should be sought.				

APPENDIX II (Cont.)

SYMPTOM	GRADE 1	GRADE 2	GRADE 3	GRADE 4
Visual	None	Blurriness, diplopia, or horizontal nystagmus of < 1 hour duration, with spontaneous resolution	> = 1 episode of Grade 2 symptoms per week, or an episode of Grade 2 Sx lasting 1 hour with spontaneous resolution by 4 hours or vertical nystagmus	Decrease in visual acuity, visual field deficit, or oculogyric crisis, or Grade 3 Sx which persist after dose reduction
Many of the symptoms in this category can be the result of CNS pathology, or alternatively can be an external (i.e., non-CNS) neuro-ophthalmologic disorder. Appropriate diagnostic investigations should be pursued.				
Myelopathy	None	None	None	Myelopathic/spinal cord symptoms, such as: Pyramidal tract weakness and disinhibition, sensory level, loss of proprioception, bladder/bowel dysfunction
HIV can cause spinal cord syndromes rarely in children. Other infectious agents can cause myelopathies as well. Alternative explanations should be sought.				
PERIPHERAL NERVOUS SYSTEM				
Neuropathy/ Lower Motor Neuronopathy	None	Mild transient Paresthesia only	Persistent or progressive paresthesias, burning sensation in feet, or mild dysesthesia; no weakness; mild to moderate deep tendon reflex changes; no sensory loss	Onset of significant weakness, decrease or loss of DTRs, sensory loss in "stocking glove" distribution, radicular sensory loss, multiple cranial nerve involvement; bladder or bowel dysfunction, fasciculations, respiratory embarrassment from chest wall weakness. Grade 3 symptoms which do not resolve with dose reduction
Infectious agents other than HIV can precipitate a neuropathy and should be considered, especially CMV. Neuropathies which do not resolve after dose reduction or discontinuation should be pursued for alternative infectious or non-infectious etiologies, since drug-related neuropathies will usually resolve after dose reduction or drug discontinuation. It should be borne in mind that many subjects will worsen for up to one month after drug discontinuation prior to improvement ("coasting"). Abnormalities should be confirmed by nerve conduction studies (NCS) +/- electromyographic studies (EMG).				
Myopathy or Neuromuscular Junction Impairment	Normal or mild (<2 x N) CPK elevation	Mild proximal weakness and/or atrophy not affecting gross motor function. Mild myalgias, +/- mild CPK elevation (<2 x N)	Proximal muscle weakness and/or atrophy affecting motor function +/- CPK elevation; or severe myalgias with CPK >2 x N; Consider confirmatory EMG and/or muscle bx	Onset of myasthenia-like symptoms (fatigable weakness with external, variable ophthalmoplegia and/or ptosis), or neuromuscular junction blockade (acute paralysis) symptoms (confirm with EMG); or Grade 3 symptoms which do not resolve on dose adjustment; confirm with muscle bx
HIV can produce a myopathy, and should be differentiated. Drug-induced myopathy can be accompanied by normal CPK levels. On occasion, neuropathic or central weakness can mimic myopathic weakness.				

APPENDIX II (Cont.)

SYMPTOM	GRADE 1	GRADE 2	GRADE 3	GRADE 4
Clinical symptoms <i>not otherwise specified</i> in this table	No therapy; monitor condition	May require minimal intervention and monitoring	Requires medical care and possible hospitalization	Requires active medical intervention, hospitalization, or hospice care
Laboratory values <i>not otherwise specified</i> in this table	Abnormal, but requiring no immediate intervention; follow	Sufficiently abnormal to require evaluation as to causality and perhaps mild therapeutic intervention, but not of sufficient severity to warrant immediate changes in study drug	Sufficiently severe to require evaluation and treatment, including at least temporary suspension of study drug	Life-threatening severity. Requires immediate evaluation, treatment, and usually hospitalization. Study drug must be stopped immediately and should not be restarted until the abnormality is clearly felt to be caused by some other mechanism than study drug.

APPENDIX II-A SUPPLEMENTAL TOXICITY TABLE FOR GRADING SEVERITY OF CUTANEOUS/SKIN RASH/DERMATITIS ADVERSE EXPERIENCES

USE THIS APPENDIX FOR GRADING CUTANEOUS/SKIN RASH/DERMATITIS ADVERSE EXPERIENCES

GRADE 1	GRADE 2	GRADE 3*	GRADE 4*
CUTANEOUS/SKIN RASH/DERMATITIS			
Erythema, with or without pruritis	<p>A. Diffuse erythematous macular or maculopapular cutaneous eruption or dry desquamation with or without pruritis; OR typical target lesions without blistering, vesicles, or ulcerations in the lesions.</p> <p>B. Urticaria</p> <p>C. A or B with fever</p>	<p>A. Diffuse erythematous macular or maculopapular cutaneous eruption or moist desquamation with or without pruritis together with any of the following constitutional findings considered related to study drug:</p> <ol style="list-style-type: none"> 1. 5 x ULN AST, ALT or 2 x baseline if baseline > ULN. 2. fever, >39°C 3. blistering and/or vesiculation of cutaneous eruptions 4. any site of mucosal lesions; OR <p>B. angioedema; OR</p> <p>C. exfoliative dermatitis defined as severe widespread erythema and dry scaling of the skin, with generalized new superficial lymphadenopathy, and with other constitutional findings such as fever, weight loss, hypoproteinemia possibly related to study drug; OR</p> <p>D. diffuse rash and serum sickness-like reactions defined as a clinical symptom complex manifested as fever, new lymphadenopathy, edema, myalgia, and/or arthralgia; OR</p> <p>E. diffuse cutaneous eruptions, usually starting on the face, trunk or back, often with prodromal symptoms plus one of the following:</p> <ol style="list-style-type: none"> 1. cutaneous bullae, sometimes confluent with widespread sheet-like detachment of skin (<10% body surface area), (Nikolski's sign)(Stevens Johnson Syndrome, SJS) 2. two or more anatomically distinct sites of mucosal erosion or ulceration not due to another cause. 	Diffuse cutaneous eruptions, usually starting on the face, trunk or back, often with prodromal symptoms plus cutaneous bullae with widespread sheet-like detachment of skin (>10% of body surface area), (Nikolski's sign), (SJS/Toxic Epidermal Necrolysis (TEN) overlap syndrome; TEN)
*When a ≥ Grade 2 cutaneous/skin rash/dermatitis adverse experience is suspected, a Dermatology consult is required.			

APPENDIX III. ADVERSE EXPERIENCE REPORTING GUIDELINES

These guidelines were compiled using the Code of Federal Regulations, the International Conference on Harmonization Guidelines, and from experience gained through previous reporting of adverse experiences and dialogue with the Pharmaceutical and Regulatory Affairs Branch of the Division of AIDS.

1.0 ADVERSE EXPERIENCE DEFINITIONS

The following definitions are compiled from 21 CFR 312.32(a) (*final rule effective April 6, 1998*) and the ICH (International Conference on Harmonisation) Technical Requirements for Registration of Pharmaceuticals for Human Use.

Adverse experience (AE): any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

This includes any change from the participant's baseline or entry status, such as:

- an onset of a new illness or medical condition since enrollment, or
- an increase in severity, frequency or rate of change of a preexisting abnormality.

Associated with the use of the study product: there is a reasonable possibility that the experience may have been caused by the study product (also called "related to study product").

Life-threatening AE: Any adverse experience that places the patient or subject at *immediate* risk of death from the reaction as it occurred. It does not include a reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse experience: Any adverse experience occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- persistent or significant disability/incapacity
- a congenital anomaly/birth defect
- important medical event that, based upon appropriate medical judgment, may jeopardize the patient or subject and may require intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization. **Note: Any adverse experience otherwise judged as serious by the on-site clinician should be reported as a serious AE.**

Severity: the clinician's evaluation of the **intensity** of the AE. Grading of severity is determined by comparing observed symptoms and/or laboratory values to those included in the Division of AIDS Tables for Grading Severity of Adverse Experiences, included Appendix II. *Note that severity is not the same as seriousness:* The term "severity" refers to the intensity of a specific event (e.g. mild, moderate or severe myocardial infarction); the event itself may be of relatively minor medical significance such as a severe headache. The term "serious", however, is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning.

Unexpected adverse experience: an AE, the specificity or severity of which is not consistent with the current investigator brochure; or if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed cerebral vascular accidents. "Unexpected", as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

2.0 AE REPORTING REQUIREMENTS

2.1 All Adverse Experiences (Serious and Non-serious)

All *non-serious* adverse experiences in mothers and infants, regardless of relatedness or expectedness to the study product, that occur before 6 weeks post-partum must be documented on the Illness/Adverse Experience case report form. Non-serious adverse experiences that occur after 6 weeks post-partum should be noted in the participant's chart but do not require a separate case report form. All *serious* AEs must be documented on the Illness/Adverse Experience case report form and reported to the PI as described below for the entire duration of the study.

Description of Adverse Experiences

The name or description of the AE should be written clearly in the space provided on the Illness/Adverse Experience case report form. *When possible, a diagnosis rather than symptoms or signs should be reported.* For example, if the participant presents with influenza, "influenza" should be recorded rather than each symptom separately (such as nausea, vomiting and diarrhea). If no diagnosis is made, record each additional symptom on a supplemental form. If more than one adverse experience is identified during the same evaluation, each should be recorded on a separate Illness/AE Form (unless they are signs or symptoms attributable to a single diagnosis, as stated above).

Severity of Adverse Experiences

The DAIDS Table for Grading Severity of Adverse Experiences ("Tox Table") is included in Appendix II as a guide to identify and grade AEs. A Guide for Estimating Severity, included

with the Tox Table, should be used to grade those clinical abnormalities which do not appear on the Tox Table.

Abnormalities at Baseline/Enrollment

For participants who enter a study with documented pre-existing abnormalities, an AE should be reported if:

- the severity increases a full grade level or more over baseline;
- the severity increases to a reportable level using the Guide for Estimating Severity from the DAIDS Tox Table;
- the participant's condition becomes serious, in the opinion of the clinician, due to the increasing severity of the abnormality.

All pre-existing abnormalities should be recorded in the patient's medical chart.

Follow-up Information

A new Illness/Adverse Experience case report form is **not** required when submitting follow-up information for a previously reported AE. The existing case report form should be updated and resubmitted. Abnormal lab results for tests done to verify or further diagnose an AE that was previously reported should not be reported as a new AE. This information should be added to comments section of the original case report form.

Patient/Subject Identification: If an infant of an enrolled mother is not born alive, the patient is considered the mother and the adverse experience (e.g. stillbirth) should be reported as her experience. If the infant is born alive (even if s/he lives only very briefly), the infant is considered the patient and the adverse experience should be reported as the infant's experience.

2.2 Serious Adverse Experiences (SAEs)

Reporting procedures for serious AEs (SAEs) are illustrated in the Serious AE Flow Chart in the Manual of Operations. All SAEs will be reported by the on-site clinician to the Principal Investigator (PI) immediately. The on-site clinician will complete the Illness/Adverse Experience case report form and fax it to the PI immediately.

- The PI will evaluate the on-site clinician's determination of the SAE's relatedness to study product and document this on the case report form. For this multi-site study, one clinician will be designated to make the assessment of relatedness, as stated in the study Manual of Operations.
- The PI will report SAEs that are possibly, probably or definitely related to study participation to FHI, the NIAID medical officer, and the PRAB Safety Specialist within 24 hours of notification by faxing the Illness/Adverse Experience case report form.
- FHI will fax the Illness/AE form to the PI of the other participating site within 48 hours of notification.

- Adverse events will be reported to the relevant IRBs and data safety and monitoring boards as required by each institution.

3.0 DOCUMENTATION

Illness/Adverse Experience Form

This form will be described in the study Manual of Operations.

Serious Adverse Experience Log

Family Health International will keep a log documenting all notifications of SAEs (AE number, date of occurrence, date identified, date notification was received by the Principal Investigator, and date the SAE was reported to FHI, DAIDS, and PRAB). This log will be reconciled with study CRFs at periodic monitoring visits.

APPENDIX IV. SAMPLE INFORMED CONSENTS

1.0 SCREENING INFORMED CONSENT

2.0 ENROLLMENT INFORMED CONSENT

1.0 SCREENING INFORMED CONSENT

Sample Informed Consent for trials sponsored by the Division of AIDS, NIAID, NIH

REMINDER TO RESEARCH SITES: DO NOT USE PREAMBLE IN LOCAL CONSENTS

NOTE FROM OPRR (OFFICE FOR PROTECTION FROM RESEARCH RISKS) TO SITES ENROLLING SUBJECTS IN THIS STUDY:

PLEASE NOTE THAT THIS SAMPLE LANGUAGE DOES NOT PREEMPT OR REPLACE LOCAL IRB REVIEW AND APPROVAL. INVESTIGATORS ARE REQUIRED TO PROVIDE THE LOCAL IRB WITH A COPY OF THIS SAMPLE CONSENT ALONG WITH THE LANGUAGE INTENDED FOR LOCAL USE. LOCAL IRBS ARE REQUIRED TO WEIGH THE UNIQUE RISKS, CONSTRAINTS, AND POPULATION CONSIDERATIONS AS A CONDITION OF ANY APPROVAL. ANY DELETION OF SUBSTANTIVE CHANGE OR INFORMATION CONCERNING RISKS OR ALTERNATIVE TREATMENTS MUST BE JUSTIFIED BY THE INVESTIGATOR, APPROVED BY THE LOCAL IRB AND NOTED IN THE IRB MINUTES. JUSTIFICATION AND IRB APPROVAL OF SUCH CHANGES MUST BE FORWARDED TO FHI FOR ANY IMC-SPONSORED TRIAL. SPONSOR-APPROVED CHANGES IN AN IMC-PROTOCOL MUST BE APPROVED BY THE LOCAL IRB BEFORE USE UNLESS INTENDED FOR THE ELIMINATION OF APPARENT IMMEDIATE HAZARD. NEW INFORMATION SHALL BE SHARED WITH EXISTING SUBJECTS AT NEXT ENCOUNTER, WITH ALL NEW SUBJECTS PRIOR TO INVOLVEMENT, OR AS THE LOCAL IRB MAY OTHERWISE ADDITIONALLY REQUIRE.

PHASE III TRIAL OF ANTIBIOTICS TO REDUCE CHORIOAMNIONITIS-RELATED PERINATAL HIV TRANSMISSION, HIVNET 024, version 1.0

SCREENING INFORMED CONSENT FORM

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Principal Investigator
University of Alabama at Birmingham
560 Old Hilman Building
618 South 20th Street
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(205)934-3273

Taha Taha, MD, PhD
Co-Principal Investigator
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615 North Wolfe Street, Room E-6007
Baltimore, MD 21205
(410)614-5255

INTRODUCTION:

You are being asked to take part in the screening for the research study named above, because you are pregnant. This study will use three drugs approved to fight infection. Before you decide whether or not to take part in the screening for this study, we would like to explain to you the purpose of the study, any risks to you, and what is expected of you.

This informed consent document gives you information about the screening visit part of the study that will be discussed with you. Once you understand the study, and if you agree to take part in the screening, you will be asked to sign this consent or make your mark in front of someone. You will be given a copy to keep.

Please note that:

- a. Your participation in this research is entirely voluntary;
- b. You may decide not to take part or to withdraw from the study at any time without losing the benefits of your standard medical care.

PURPOSE OF THE STUDY:

The purpose of this research study is to see if drugs given to treat an infection of the uterus during pregnancy can reduce the chances of HIV, the virus that causes AIDS, being passed from an HIV-positive mother to her baby. The study will also see if these drugs improve the birthweight of the baby or prevent delivering the baby too early in an HIV positive or an HIV negative woman. Three drugs will be used in this study. These drugs have been approved for the treatment of infection of the uterus. Many women have used these drugs. Some studies have shown that an infection of the uterus during pregnancy is associated with a mother with HIV passing the virus to her baby. None of these drugs being given are to treat HIV infection.

This research study will enroll about 1300 women at this site and about 1300 women at a site in (Zambia/Malawi). Participation in the study will last about 1 year.

If you are interested in volunteering for this research study, you will need to meet the requirements for enrollment. You are being asked to give your permission to be tested for HIV and to see if you can be in the study.

If you meet the study requirements, you will be given more information about the study in another consent form. If you agree to participate in the study you will be asked to sign or make your mark on that consent form.

PROCEDURES:

After you sign this consent form, you will be asked to give a blood sample. About two teaspoons of blood will be needed. Your blood sample will be tested to see if you are infected with HIV or syphilis. Your test results will be available [today (Zambia)/ in one week (Malawi)]. (In Malawi: You will be asked to return to the clinic in one to two weeks to get your test results.) Your test results will be explained to you. If you want to be in this research study, you must receive your HIV test results.

If your syphilis test shows that you have syphilis, you will be offered treatment at this clinic. You will not have to pay for this treatment.

You will be asked questions about any previous pregnancies and your medical history. Based on the results of your HIV test and your pregnancy and medical history, you may be asked to be in the study.

If your HIV test result is not positive but it also not negative, you will not be able to be in this study. This type of test result means that doctors can not tell whether you are or are not infected with HIV.

RISKS and/or DISCOMFORTS:

Some people experience discomfort when blood is drawn and may feel dizzy or even faint. You may have a bruise or swelling where the needle goes into your arm.

If you have HIV, knowing your HIV status may cause you anxiety. If others find out your HIV status, you may have trouble finding or keeping a job and have problems being accepted in your family and community.

POTENTIAL BENEFITS:

You may receive no direct benefit from these tests. However, you will receive information about your HIV status.

COSTS TO YOU:

There is no cost to you for these tests.

CONFIDENTIALITY:

Your research records will be confidential to the extent permitted by law. You will be identified by a code, and personal information from your records will not be released without your written permission. You will not be personally identified in any publication about this study.

Your records may be reviewed by the National Institutes of Health, the U.S. agency that sponsors this research, and the study monitors.

RESEARCH-RELATED INJURY:

If you are injured as a result of participation in this screening, the study clinic will give you immediate necessary treatment for your injuries. The cost of this treatment will not be charged to you. You will then be told where you may receive additional treatment for injuries. There is no compensation provided for research-related injuries.

NOTE: You are not giving up any of your legal rights by signing this informed consent document.

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS:

If you ever have questions about this screening or in case you are injured as a result of participation in this screening, you should contact (name of local investigator or study clinician) at (telephone number or address). If you ever have questions about your rights as a research subject you may call (name and title of IRB member) at (telephone number or address).

SIGNATURE PAGE:

If you have read the informed consent, or had it read and explained to you, and you understand the information and voluntarily agree to be screened to join this study, please sign your name or make your mark below.

Volunteer's name Volunteer's signature Date

Witness' name Witness' signature Date

2.0 ENROLLMENT INFORMED CONSENT

Sample Informed Consent for trials sponsored by the Division of AIDS, NIAID, NIH

REMINDER TO RESEARCH SITES: DO NOT USE PREAMBLE IN LOCAL CONSENTS

NOTE FROM OPRR (OFFICE FOR PROTECTION FROM RESEARCH RISKS) TO SITES ENROLLING SUBJECTS IN THIS STUDY:

PLEASE NOTE THAT THIS SAMPLE LANGUAGE DOES NOT PREEMPT OR REPLACE LOCAL IRB REVIEW AND APPROVAL. INVESTIGATORS ARE REQUIRED TO PROVIDE THE LOCAL IRB WITH A COPY OF THIS SAMPLE CONSENT ALONG WITH THE LANGUAGE INTENDED FOR LOCAL USE. LOCAL IRBS ARE REQUIRED TO WEIGH THE UNIQUE RISKS, CONSTRAINTS, AND POPULATION CONSIDERATIONS AS A CONDITION OF ANY APPROVAL. ANY DELETION OF SUBSTANTIVE CHANGE OR INFORMATION CONCERNING RISKS OR ALTERNATIVE TREATMENTS MUST BE JUSTIFIED BY THE INVESTIGATOR, APPROVED BY THE LOCAL IRB AND NOTED IN THE IRB MINUTES. JUSTIFICATION AND IRB APPROVAL OF SUCH CHANGES MUST BE FORWARDED TO FHI FOR ANY IMC-SPONSORED TRIAL. SPONSOR-APPROVED CHANGES IN AN IMC-PROTOCOL MUST BE APPROVED BY THE LOCAL IRB BEFORE USE UNLESS INTENDED FOR THE ELIMINATION OF APPARENT IMMEDIATE HAZARD. NEW INFORMATION SHALL BE SHARED WITH EXISTING SUBJECTS AT NEXT ENCOUNTER, WITH ALL NEW SUBJECTS PRIOR TO INVOLVEMENT, OR AS THE LOCAL IRB MAY OTHERWISE ADDITIONALLY REQUIRE.

PHASE III TRIAL OF ANTIBIOTICS TO REDUCE CHORIOAMNIONITIS-RELATED PERINATAL HIV TRANSMISSION, HIVNET 024, version 1.0

ENROLLMENT INFORMED CONSENT FORM

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Taha Taha, MD, PhD
Co-Principal Investigator
Johns Hopkins University
School of Hygiene and Public Health
615 North Wolfe Street, Room E-6007
Baltimore, MD 21205
(410)614-5255

INTRODUCTION:

You are being asked to take part in the research study named above, because you are pregnant. This study will use three drugs approved to fight infection. Before you decide whether or not to take part in this study, we would like to explain to you the purpose of the study, any risks to you, and what is expected of you.

This informed consent document gives you information about the study which will be discussed

with you. Once you understand the study, and if you agree to take part, you will be asked to sign this consent or make your mark in front of someone. You will be given a copy to keep.

Please note that:

- a. Your participation in this research is entirely voluntary;
- b. You may decide not to take part or to withdraw from the study at any time without losing the benefits of your standard medical care.
- c. If your baby's father refuses to allow you to be in the study, you and your baby cannot be in the study.

PURPOSE OF THE STUDY:

The purpose of this research study is to see if drugs given to treat an infection of the uterus during pregnancy can reduce the chances of HIV, the virus that causes AIDS, being passed from an HIV-positive mother to her baby. The study will also see if these drugs improve the birthweight of the baby or prevent delivering the baby too early in an HIV positive or an HIV negative woman. Three drugs, metronidazole, erythromycin, and ampicillin, will be used in this study. These drugs have been approved for the treatment of infection of the uterus. Many women have used these drugs. Some studies have shown that an infection of the uterus during pregnancy is associated with passing HIV from an HIV positive mother to her baby. None of the drugs given are to treat HIV infection.

(In Malawi: A study in Thailand showed that AZT given to pregnant women during the last four weeks of pregnancy and during labor reduced the chances of passing HIV from an HIV-positive mother to her baby by 50%. Women in the study in Thailand did not breastfeed. It is not known how much giving AZT during pregnancy and delivery would reduce the chances of an HIV positive woman passing the virus to her baby during breastfeeding. AZT is not routinely available currently in Malawi.)

This research study will enroll about 1300 women at this site and about 1300 women at a site in (Zambia/Malawi). Participation in the study will last about 1 year.

PROCEDURES:

After you sign the informed consent and have had a chance to ask questions, you will have three study visits before you go into labor. At this first visit you will be asked about your health and any medications you have taken recently. You will be asked questions about your sexual history. Some people may be embarrassed by these questions. You may choose not to answer any of the questions if you wish. You will have a pelvic exam (an examination of your vagina) and will be tested for sexually transmitted diseases. You will have about 2 teaspoons of blood drawn for tests. You will be given vitamins to take by mouth every day until delivery.

At this visit you will be assigned by chance (like the tossing of a coin) to take the study drugs or placebo. A placebo is a pill that looks just like the study drug but has no medicine in it. One woman of every two will receive study drugs. One woman of every two will receive placebo. The study doctors and you will not know if you are taking the study drug or placebo. Both groups of women, those assigned the study drugs (metronidazole and erythromycin) and those

assigned the placebo will take their pills by mouth three times a day for 7 days. You will be asked to bring your pill packs, even if they are empty, to your next study visit.

At your second visit, you will be asked questions about your health and any medications you have taken since the last visit. You will have a pelvic exam and will be tested for sexually transmitted diseases. Your pill packs given to you at the first visit will be collected from you.

At your third visit you will be asked about your health and any medications that you have taken since the last visit. You will be given pill packs to take home with you for when you go into labor.

When you go into labor, you will again begin taking either study drugs or placebo as assigned. Women assigned study drugs (metronidazole and ampicillin) and placebo will take pills by mouth every four hours until the baby is delivered. You will return to the hospital to deliver your baby. You will be asked about medications you have taken since your last visit. You will have about 2 teaspoons of blood drawn for tests. After delivery, your placenta and cord will be tested to see if you had an infection during delivery. A sample of your breast milk will be collected. If you have HIV, your breast milk will be stored for future tests. Your baby will be weighed and examined. Your baby will have a heelstick to get a few drops of blood. If you have HIV, your baby's blood will be tested for HIV.

After delivery, you will continue taking the study drugs or placebo 3 times a day until you do not have any more drugs. You will bring your pill packs to your next clinic visit, even if they are empty.

You and your baby will return for 5 study visits during the year after your baby is born. At each visit you will be asked questions about your health, your baby's health, and your breastfeeding practices. At three of the visits your baby will have a heelstick to get a few drops of blood. If you are HIV positive, your baby's blood will be tested for HIV. The results of your baby's HIV test will be given to you. You must agree to receive your baby's HIV test results to be in the study.

If you do not deliver your baby at the hospital, you will still be asked to continue study visits so that the research staff can gather more information which may be useful and important for future subjects.

Some of your blood and tissue will be stored for later tests. Some of your placenta will be stored for later tests. Your name will not be linked to these samples. They will be identified by a code to protect your privacy.

You and your baby will receive all of your standard medical care as part of this study. You should tell your study nurse or doctor when taking any non-study medications or enrolling in other research studies.

All pills given to you should be kept at room temperature away from heat and light. If you miss a dose, take it as soon as possible unless it is almost time for the next dose. If it is almost time

for the next dose, then skip the missed dose and go back to your regular dosing schedule. Do not take two doses at once.

RISKS and/or DISCOMFORTS:

All three antibiotics have been used and studied in pregnant women. Some women are allergic to the antibiotics. Allergic means you have a certain kind of reaction after taking the drug. A very small number of people who take each of these drugs have serious reactions. If you experience any of the following, you should stop taking the pills and contact the study clinic immediately:

- skin rash;
- vomiting;
- severe cramping;
- tightness of chest;
- swelling of eyelids, face or lips;
- wheezing or difficulty breathing.

Rarely, people taking these antibiotics have numbness or tingling in their arms and legs, seizures, and other nervous disorders. If you experience any of these side effects, you should stop taking the pills and contact the study clinic immediately.

If the medicine upsets your stomach, take your _____ (*specify color of erythromycin capsule*) pill two times a day instead of three for 2-3 days. If the medicine still upsets your stomach, take your _____ (*specify color of erythromycin capsule*) pill one time a day for 2-3 days. Continue taking your medicine until it is gone.

Do not drink alcohol while taking the pills. This may make you feel very sick. Wait for at least 3 days after you have stopped taking the pills to drink any alcohol.

There may be some more risks from taking non-study drugs with the study drugs. There may be risks to your baby from taking these drugs, but no risks are known.

Some women experience mild discomfort during a pelvic exam. Some people experience discomfort when blood is drawn and may feel dizzy or even faint. You may have a bruise or swelling where the needle goes into your arm.

POTENTIAL BENEFITS:

By taking part in this research study, you will receive vitamins to take during pregnancy that may help you and your baby. Being in this study may reduce the chance of having your baby too soon and may help the overall health of your baby, but no guarantee can be made. If you have HIV, taking part in this study may reduce the chance of your baby being infected with HIV, but no guarantee can be made. You may receive no benefit from this study. However, knowledge gained from this study may help others in the future.

NEW FINDINGS:

You will be told of any new information learned during the course of the study that might cause you to change your mind about staying in the study.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY WITHOUT YOUR CONSENT:

You may be removed from the study without your consent for the following reasons:

- a. the study doctor decides that continuing in the study would be harmful to you;
- b. the study is cancelled by the United States National Institutes of Health (NIH)
- c. other administrative reasons.
- d. your baby's father withdraws his permission for your baby's participation in the study.

If you need a treatment not allowed on this study or you have a bad reaction to the study drugs you will stop taking the study drugs, but you and your baby will continue to have follow-up study visits.

ALTERNATIVES TO PARTICIPATION:

(In Malawi: There are no drugs currently available to women in Malawi to help prevent an HIV-positive mother from passing HIV to her baby.)

(In Zambia: A study in Thailand showed that AZT given to pregnant women during the last four weeks of pregnancy and during labor reduced the chances of passing HIV from an HIV positive mother to her baby by 50%. Women in the study in Thailand did not breastfeed. It is not known how much giving AZT during pregnancy and delivery would reduce the chances of passing the virus from a mother with HIV to her baby during breastfeeding. AZT is only available at some private clinics but is very expensive. The goal of this study is to find an inexpensive way to reduce infections and the chances of a mother with HIV passing HIV to her baby that could be used with all pregnant women in Zambia. However, if you think you can afford AZT yourself or can get it through other means, we can refer you to an appropriate private clinic where you can buy it. Because the effect of the antibiotics in our study may not be detectable if AZT is used, women who take AZT during their pregnancy are not eligible for this trial.)

If you do not participate in this study, you and your baby will receive standard medical care at this clinic/hospital.

Before you decide to take part in this study, your study clinician will give you information about the risks and any potential benefits of participating in this research study.

COSTS TO YOU:

There is no cost to you for participating in the study. All of your drugs and study visits are free.

CONFIDENTIALITY:

Your research records will be confidential to the extent permitted by law. You will be identified by a code, and personal information from your records will not be released without your written permission. You will not be personally identified in any publication about this study.

Your records may be reviewed by the U.S. agency that reviews research, the agency that sponsors this research, and the study monitors.

RESEARCH-RELATED INJURY:

If you are injured as a result of participation in this study, the study clinic will give you immediate necessary treatment for your injuries. The cost of this treatment will not be charged to you. You will then be told where you may receive additional treatment for injuries. There is no compensation provided for research-related injuries.

NOTE: You are not giving up any of your legal rights by signing this informed consent document.

PERSONS TO CONTACT FOR PROBLEMS OR QUESTIONS:

If you ever have questions about this study or in case you are injured as a result of participation in this research study, you should contact (*name of local investigator or study clinician*) at (*telephone number or address*). If you ever have questions about your rights as a research subject you may call (*name and title of IRB member*) at (*telephone number or address*).

SIGNATURE PAGE

If you have read the informed consent or had it read and explained to you and understand the information, and you voluntarily agree to join this study, please sign your name or make your mark below.

Volunteer's name Volunteer's signature Date

Witness' name Witness' signature Date

If reasonably available:

Father's name Father's signature Date

**APPENDIX V. “CHORIODECIDUAL INFLAMMATION: A POTENTIALLY
PREVENTABLE CAUSE OF PERINATAL HIV TRANSMISSION?”**

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Choriodecidual Inflammation:
A Potentially Preventable Cause of Perinatal HIV Transmission?

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Abstract

The obstetric risk factors for perinatal HIV transmission include preterm birth, prolonged rupture of the chorioamniotic membranes, and both clinical and histologic bacterial chorioamnionitis. A chronic chorioamnionitis appears to precede many cases of preterm labor and spontaneous rupture of membranes, while an acute chorioamnionitis is more common following rupture of the membranes at term. Amniotic fluid cytokines are elevated in the presence of both term and preterm intrauterine bacterial infections, and various cytokines appear able to attract HIV-infected white cells into the amniotic cavity and to increase HIV replication. We hypothesize that the association of preterm birth and prolonged rupture of membranes with perinatal HIV transmission may often be due to an associated chronic or acute bacterial chorioamnionitis marked by the migration of HIV-infected maternal white cells into the amniotic cavity. This sequence of events leading to maternal-infant HIV transmission may be prevented by antibiotic treatment.

Key words: antibiotics, zidovudine, chorioamnionitis, HIV, perinatal transmission, preterm birth, ruptured membranes

Introduction

Transmission of HIV from mother to fetus is the major cause of childhood AIDS. Transmission occurs in 15 to 40% of HIV-positive maternal/fetal pairs in the absence of zidovudine (ZDU) prophylaxis and approximately 5-10% with prophylaxis.¹ The regimen recommended by the CDC is likely to cost about \$1500 per pregnancy; a shorter course of ZDU proximal to delivery may cost \$100. In many developing countries, because of the high cost per woman treated and the large numbers of HIV-positive pregnant women, it is unlikely that widespread use of antiretroviral therapy will soon occur. Therefore, other strategies, both low-cost and practical, must be developed if transmission is to be reduced in the developing world.

Risk Factors for Perinatal Transmission

The most potent predictors of perinatal HIV transmission are viral load and maternal disease severity. However, without antiretroviral therapy, lowering maternal viral load or reducing disease severity is not a viable option. Obstetric factors related to transmission include 1) preterm birth, with the earlier the birth, the higher the rate,² 2) histologic and clinical chorioamnionitis,³⁻⁵ and 3) prolonged rupture of membranes (ROM).⁶ Other risk factors for transmission include various STDs, first versus second twin, maternal bleeding, smoking, and obstetric procedures including forceps delivery, scalp electrode placement, and amniocentesis. However, preterm birth, chorioamnionitis, and prolonged ROM appear to be the strongest and most consistent obstetric risk factors for transmission.

Mechanisms of Transmission

Many questions related to perinatal HIV transmission remain unanswered. Most prominent are the route and timing of transmission.⁷ Based upon finding virus in fetal tissues in each trimester, transmission may occur throughout pregnancy. However, because of the delayed appearance of HIV in many newborns, it is believed that 1) the majority of transmissions occur during labor and delivery, 2) a minority occur prenatally, and 3) additional transmission occurs with breast feeding. The mechanism(s) by which the fetus becomes infected remains uncertain. Transmission may occur following fetal exposure to 1) HIV infected vaginal fluid, 2) HIV infected amniotic fluid, 3) maternal blood across the placenta, and 4) maternal blood during delivery. Any of these pathways are possible, especially since both free virus and HIV infected white cells have been found in both vaginal and amniotic fluid,^{8,9} and virus has been demonstrated in maternal blood and placental tissue. The contribution of each pathway to transmission is unknown.

Hypothesis

Consideration of the obstetric risk factors provides a clue to the most important routes, and a potential strategy, for reducing transmission. First, we now believe that chorioamnionitis may be the major obstetric risk factor for HIV transmission, and that the increased risk associated with preterm birth and prolonged ROM is explained by chorioamnionitis associated with these conditions. (Fig. 1) We emphasize that chorioamnionitis is an inflammation/infection of placental membranes. Less evidence exists that placental inflammation is associated with transmission.

Preterm Birth

Preterm birth, and especially early preterm birth, is strongly associated with chronic chorioamnionitis due to organisms such as *Mycoplasma*, *Ureaplasma*, *Bacteroides*, and *Gardnerella*.¹⁰ Histologic chorioamnionitis associated with these organisms is found in 80% or more of women delivering at less than 30 weeks' gestation. More than 50% of infants born to HIV-positive women at less than 30 weeks' gestation may be HIV-positive.² While these infants may be more susceptible to HIV infection because of reduced defenses and an immature immunologic system, increased transmission may also be associated with pre-existing bacterial chorioamnionitis. Further evidence for this hypothesis is found in a study by Nyong'o et al, in which histologic chorioamnionitis was associated with HIV transmission in preterm but not term deliveries.⁵ These differences might occur because chorioamnionitis associated with preterm birth is more likely to be chronic, allowing more time for transmission. Chronic chorioamnionitis also tends to have mononuclear rather than polymorphonuclear responses, with mononuclear cells more likely to harbor HIV.¹¹

Substantial evidence exists that most early preterm birth is associated with a chronic intrauterine infection present weeks or months before delivery.¹⁰ Evidence includes 1) elevated amniotic fluid cytokines at 16 weeks in women who eventually deliver as late as 32 weeks, 2) *Ureaplasma* in amniotic fluid 6-8 weeks before labor, 3) markers of intrauterine infection such as fetal fibronectin in the cervix 7-8 weeks before labor and the development of chorioamnionitis,¹² and 4) documented chorioamnionitis in women in preterm labor before ROM.¹⁰

There is a clear relationship between the presence of bacterial vaginosis, an overgrowth of

vaginal organisms including *Ureaplasma*, *Mycoplasma*, and *Gardnerella*, and spontaneous preterm birth.¹⁰ Women with bacterial vaginosis also develop more chorioamnionitis. In fact, we now believe that bacterial vaginosis is a “marker” for bacterial chorioamnionitis. Targeting this marker, preterm birth has been substantially reduced with a prolonged course of metronidazole with or without erythromycin.^{10,13} Also, substantial reductions in preterm birth have followed treatment of women in preterm labor with metronidazole and ampicillin, presumably by successfully treating a subclinical chorioamnionitis.¹⁴

Large inter-country differences in perinatal transmission rates were reported even prior to the widespread use of antiretroviral agents in developed countries. In France, the transmission rate was 14%, while in sub-Saharan Africa, transmission rates of 30 to 40% were common. Breast feeding alone does not explain these differences. Various STDs, including syphilis and gonorrhea, while associated with transmission in some studies, do not explain the high rates in African women, nor do they explain the increased prematurity rate in U.S. black women.¹⁵ However, bacterial vaginosis is increased in black versus white women in both the U.S. and Great Britain (30-40% versus 10-15%), and rates of bacterial vaginosis in sub-Saharan Africa are often reported as 30% to 50%. The attributable risk of bacterial vaginosis to preterm birth in U.S. black populations approaches 40%.¹⁶ If bacterial vaginosis-related chorioamnionitis explains some of the difference in prematurity rates between black and white U.S. populations, it may also explain part of the difference in HIV transmission between European and African populations. Of major importance to this hypothesis, Taha et al recently reported that Malawian women with bacterial vaginosis were twice as likely to transmit HIV to their infants (28 vs. 14%) as other women.¹⁷

Prolonged Rupture of Membranes

The association of prolonged ROM with transmission may be explained by prolonged fetal exposure to HIV infected vaginal fluid. Another explanation may be the increased risk of chorioamnionitis.⁶ The association between prolonged ROM and chorioamnionitis stems from two factors. First, preexisting chorioamnionitis likely causes ROM. Second, the longer the ROM, the more likely virulent vaginal bacteria, such as group B streptococci, will ascend and cause chorioamnionitis. In any case, it appears that the ROM-associated chorioamnionitis is related to HIV transmission. Evidence for this hypothesis includes a study by Popek et al.,⁴ which demonstrated that 1) with ROM less than four hours and without chorioamnionitis, transmission was 6%, 2) with ROM less than four hours with chorioamnionitis, transmission was 6%, 3) with ROM four hours or more without chorioamnionitis, transmission was 5%. 4) However, with ROM of four or more hours with chorioamnionitis, transmission was 38%. This study strongly suggests that it is not ROM alone which is associated with transmission, but prolonged ROM and chorioamnionitis.

Biggars et al reported no impact of vaginal microbicides on HIV transmission, except with ROM of four hours or more.¹⁸ One interpretation of these results is that with ROM, microbicides required four hours to be effective. Alternatively, chorioamnionitis due to ascending bacterial infection increases with prolonged ROM. Because a reduction in chorioamnionitis using vaginal microbicides has been demonstrated, it is possible that microbicides reduced the incidence of chorioamnionitis associated with prolonged ROM, resulting in less HIV transmission. Whether microbicides or antibiotics will reduce chorioamnionitis-associated transmission in women with

ROM is unknown. However, in HIV-negative women with preterm ROM, antibiotic prophylaxis reduces both chorioamnionitis and neonatal sepsis.¹⁹

Cesarean Section

The studies relating cesarean section to perinatal HIV transmission have produced conflicting results.²⁰ One reason for the discrepancies is that indications for cesarean section were often not considered. For example, gestational age and duration of ROM influence transmission, but were not considered in most studies. If most cesareans are performed before labor with membranes intact, transmission rates should be low because chorioamnionitis is rare. Conversely, if cesareans follow long labors with prolonged ROM and chorioamnionitis, a reduction in transmission would be unlikely. Recent studies confirm that cesareans are protective against transmission only when performed before labor or soon after ROM, i.e., before the development of chorioamnionitis.²¹

Cytokines

Bacterial invasion of the uterus engenders a massive cytokine response by the macrophage-like cells of the decidua, placenta and membranes.¹⁰ In infected women, cytokines such as IL-6 are found in very high concentrations in the amniotic fluid. These cytokines attract large numbers of white cells (which may be HIV infected) into the amniotic fluid. Schafer also showed that HIV infected macrophages exposed to various cytokines increased HIV replication.²² Fetal exposure to the HIV-infected amniotic fluid may be responsible for the high transmission rates associated with chorioamnionitis. The feasibility of viral transmission through fetal skin or mucus

membranes has been demonstrated by injecting HIV intra-amniotically into non-infected monkeys, with most fetuses born infected.²³

Testing the Hypothesis

We hypothesize that important components of perinatal HIV transmission include both preterm chronic chorioamnionitis and acute chorioamnionitis occurring at term. We also believe a portion of this chorioamnionitis is either preventable, or treatable in its early stages using either metronidazole alone or with other antibiotics. By reducing chorioamnionitis in pregnant women, HIV transmission will be reduced.

Who to treat, and when, is debatable. Since preterm birth has been reduced with second trimester antibiotics using bacterial vaginosis as a marker,^{13,15} this strategy may reduce HIV transmission. Antibiotic treatment of women at risk for prolonged ROM and chorioamnionitis may also achieve a reduction in HIV transmission, since a similar strategy has reduced chorioamnionitis in women with ROM.¹⁹ Candidates might include women presenting with ROM and not in labor, or those who because of parity, cervical exam, size of baby or pelvis, or poor labor progress, are at risk for chorioamnionitis. We therefore believe that it would be appropriate to attempt to reduce perinatal HIV transmission in HIV-positive women using antibiotics in the second trimester and again at delivery, targeting chronic and acute chorioamnionitis. Since each trial reporting a significant reduction in preterm birth used a 7-10 day course of metronidazole and often ampicillin or erythromycin, using similar regimens in randomized trials to reduce HIV transmission would be appropriate.^{10,13} A repeat of the Malawi microbicide study, this time attempting to reduce acute chorioamnionitis at delivery, would also

be of interest.

In developed countries, targeting chorioamnionitis with antibiotics or microbicides may provide additional reductions in transmission when used with standard antiretroviral regimens. However, because long duration ZDU therapy has been successful in reducing HIV transmission,¹ additional benefits will be small. However, if beneficial in reducing transmission, the value of an inexpensive antibacterial approach is obvious, especially if antiretrovirals are not available. Instead of thousands or hundreds of dollars for antiretrovirals, the total cost of antibiotics per woman treated in sub-Saharan Africa should be less than \$10, and for the microbicides, far less. If even a small reduction in HIV transmission were achieved using antibiotics, the cost per transmission averted would be low. If 100 women were treated both in the second trimester and in labor at a total antibiotic cost of \$10, the cost for treating 100 women would be \$1,000. If the transmission rate decreased from 35% to only 30%, (5 neonatal HIV cases per hundred women), the cost per case averted would be about \$200. This is far less than for ZDU therapy, in which cost per case averted is approximately \$5,000 to \$10,000.¹ If short course ZDU achieved a 50% reduction in transmission as in the Thai trial,²⁴ the cost per case averted would be about \$1,100.²⁵ Therefore, an antimicrobial strategy could be effective even if no antiretrovirals were available, and may be relatively cost-effective even when compared to short-course antiretroviral therapy. Also, if in some developing countries, a late-pregnancy short-course antiretroviral strategy were adopted, antibiotic treatment aimed at reducing preterm chorioamnionitis-related HIV transmissions might nicely complement this approach.

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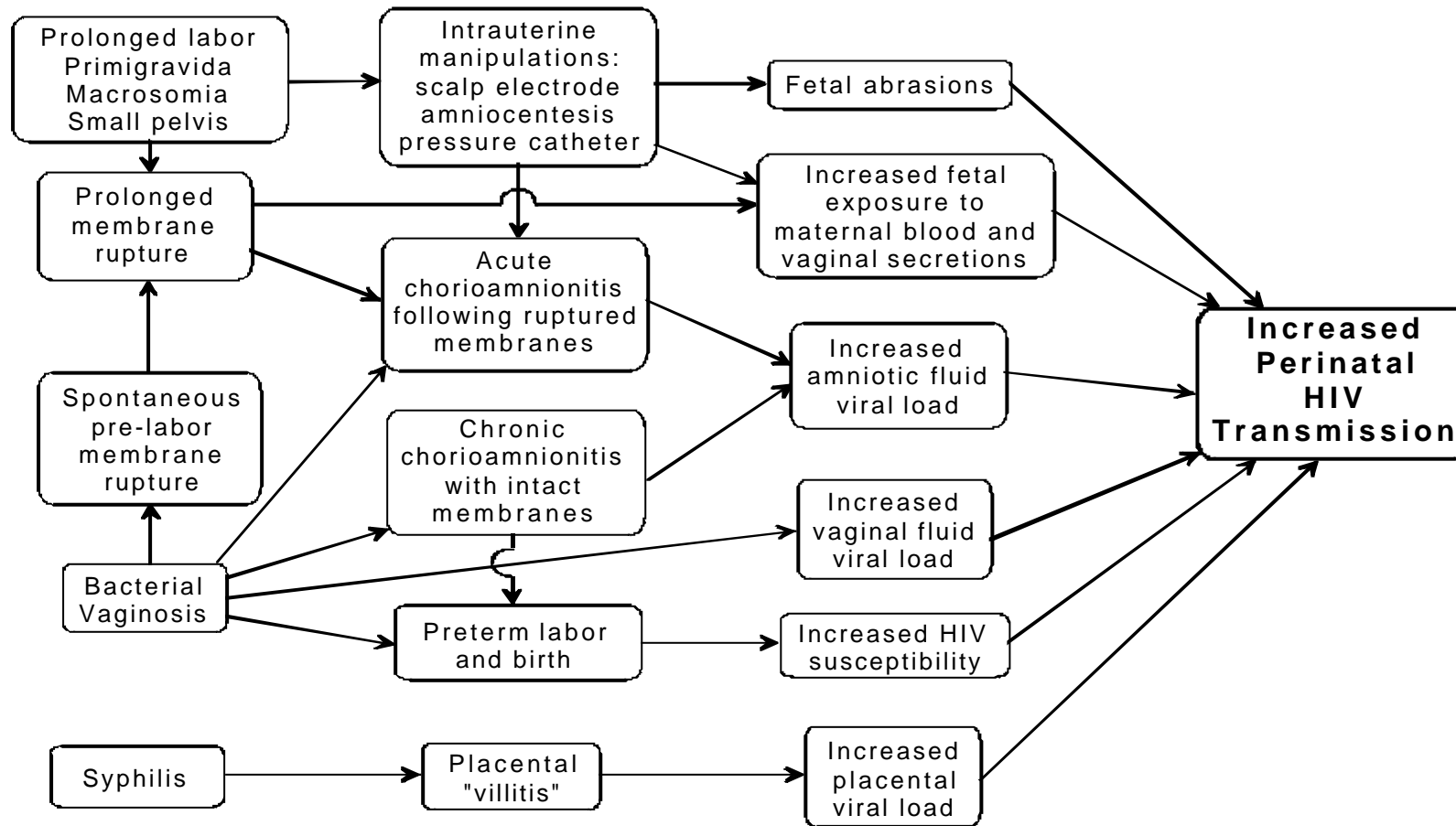
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Fig.1. A model relating bacterial vaginosis, preterm labor, membrane rupture and chorioamnionitis to perinatal HIV transmission.

POTENTIAL MECHANISMS OF PERINATAL HIV TRANSMISSION



APPENDIX VI. EVALUATION OF PLACENTA

Form I: Placental Evaluation

Participant Identification Number:

Date of Exam:

PLACENTA EXAM

Trimmed weight (gm):

CORD

Neutrophilic (N) or Monocytic (M) infiltration of umbilical vein, artery and cord substance:

Not seen Marked N/A
 Slight Unable to Determine

MEMBRANES

Neutrophilic (N) or Monocytic (M) infiltration of amion and chorion of membrane roll:

Not seen Marked N/A
 Slight Unable to Determine

DECIDUA OF PLACENTAL FLOOR AND MEMBRANES

Neutrophilic (N) or Monocytic (M) infiltration of amion and chorion of membrane roll:

Not seen Marked N/A
 Slight Unable to Determine

PLACENTAL VILLI

Neutrophilic (N) or Monocytic (M) infiltration of amion and chorion of membrane roll:

Not seen Marked N/A
 Slight Unable to Determine

PLACENTAL MALARIA FINDINGS

presence of:

malarial pigments deposits malaria parasites in the intervillous spaces
 excessive perivillous fibrinoid deposits lymphocytic infiltration into preivillous spaces

