A PROSPECTIVE, OBSERVATIONAL STUDY OF HIV-INFECTED PREGNANT WOMEN AND THEIR INFANTS AT CLINICAL SITES IN LATIN AMERICAN AND CARIBBEAN COUNTRIES

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PROTOCOL TEAM ROSTER

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PRECIS

This is an observational, prospective cohort study to describe the characteristics of HIV-infected pregnant women and their infants at participating clinical sites in Latin America and the Caribbean where formula and antiretroviral prophylaxis of mother-to-child transmission of HIV are available. We will describe the utilization of interventions related to decreasing the risk of mother-to-child transmission, including antiretroviral prophylaxis, cesarean section before labor and before ruptured membranes, and avoidance of breastfeeding. We will describe receipt of maternal antiretroviral therapies and determine mother-to-child HIV transmission rates. This study will describe maternal adverse events during pregnancy and the postpartum period. In addition, the study will describe infant outcomes potentially related to in utero and early infant exposure to antiretroviral medications and to mode of delivery. We will enroll approximately 180-240 HIV-infected pregnant women during the first year of this planned five-year study. HIV-infected women will be evaluated antepartum, intrapartum and six months postpartum. HIV-exposed infants will be evaluated through six months of age.

SCHEMA

A PROSPECTIVE, OBSERVATIONAL STUDY OF HIV-INFECTED PREGNANT WOMEN AND THEIR INFANTS AT CLINICAL SITES IN LATIN AMERICAN AND CARIBBEAN COUNTRIES

DESIGN: Prospective cohort study to describe the characteristics of HIV-infected pregnant women and their infants followed at participating Latin American and Caribbean sites, and to characterize adverse events during pregnancy and the postpartum period (HIV-infected women) and during early infancy (HIV-exposed infants) according to both receipt of/exposure to antiretroviral drug(s) and mode of delivery.

SAMPLE SIZE AND POPULATION: HIV-infected pregnant women receiving medical care at participating clinical sites who: 1) plan to deliver at the clinical site; and 2) plan to continue postpartum medical care for six months at the clinical site or associated outpatient facility may be enrolled. Infants born to enrolled women will be followed through six months of age. HIV-exposed infants also will be offered enrollment into a related pediatric cohort study. It is anticipated that approximately 180-240 women will be enrolled during the first year.

REGIMEN: HIV-infected women: Prospective data collection during the antepartum and intrapartum periods and for six months postpartum will include history and physical examination, as well as laboratory evaluations (including hematology and flow cytometry, HIV virologic assays, and biochemical assays).

Their HIV-exposed infants: Prospective data collection through ***six*** months of age will include history and physical examination, as well as laboratory evaluations (hematology and flow cytometry, HIV diagnostic assays, and biochemical assays) and assessments of growth and morbidity.

STUDY DURATION: Women will be followed through six months postpartum. Their HIV-exposed infants will be followed through six months of age.

PRIMARY OBJECTIVES: 1) To describe the characteristics of HIV-infected pregnant women and their HIV-exposed infants receiving care at participating clinical sites in Latin America and the Caribbean, including the utilization of interventions related to decreasing the risk of maternal-child transmission (antiretroviral therapy, cesarean section before labor and before ruptured membranes, avoidance of breastfeeding); the receipt of antiretroviral or other therapy for the woman's own health; and mother-to-child HIV transmission rates.

2) To characterize adverse events during pregnancy and the postpartum period (HIV-infected women) and during early infancy (HIV-exposed infants) according to both receipt of/exposure to antiretroviral drug(s) and mode of delivery.

1.0 INTRODUCTION

Efficacious and feasible interventions to prevent transmission of human immunodeficiency virus type 1 (HIV) from mother to child have been developed over the past several years. There has been a dramatic reduction in mother-to-child, or vertical, transmission of this viral infection in many countries, including those in the Americas and in the Caribbean. In addition, the medical management of HIV-infected women during pregnancy has evolved significantly. However, risks associated with such interventions and management also must be evaluated on an ongoing basis so that HIV-infected women can make informed decisions regarding their own health and that of their HIV-exposed children**.**

1.1 Background

1.11 Prevention of Mother-to-Child Transmission of HIV

By the end of 1999, the Joint United Nations Programme on HIV/AIDS (1) estimated that 1.2 million children were living with HIV infection. During 1999 alone, approximately 600,000 children were newly infected with HIV on a global basis, mostly in less-developed countries. The majority of pediatric HIV infection is acquired by transmission from mother to child during pregnancy, at birth or through breast milk; in the U.S., over 90% of pediatric HIV infection is acquired from the mother (2). Without prophylactic interventions, vertical transmission rates range from 12-42% (3), with the highest rates of transmission observed among breastfeeding populations.

In the U.S. and several other countries, the prevention of mother-to-child transmission historically has had several foci: provision of HIV counseling and testing, provision of antiretroviral prophylaxis, and avoidance of breastfeeding. Routine HIV counseling and voluntary testing for all pregnant women has been recommended (4), and more recently universal, routine testing of pregnant women with patient notification has been advocated (5). The goal of either approach would be to enable HIV-infected women to seek medical care for their own health as well as interventions to reduce the risk of transmission of HIV to their children and to their sexual partners. In 1994, administration of zidovudine (ZDV) to the mother during pregnancy after the first trimester, intravenously during labor and delivery, and to the infant for 6 weeks after birth was shown to significantly reduce mother-to-child HIV transmission in Pediatric AIDS Clinical Trials Group (PACTG) protocol 076, a clinical trial conducted in the United States and France (6). Incorporation of this complex regimen into clinical practice, coupled with increased prenatal HIV counseling and testing, has resulted in a decline in perinatal transmission rates to as low as 4-6% in the U.S. (7, 8, 9), with a concomitant significant decline in reported cases of perinatal AIDS (9). Initiation of programs including HIV counseling and testing, ZDV prophylaxis, and avoidance of breastfeeding where possible has been accompanied by similar decreases in perinatal transmission in Latin America and the Caribbean. For example, in Brazil, ZDV has been provided free of charge to HIV-infected pregnant women since 1995, and intravenous ZDV has been provided for administration during labor since 1996; several reports indicate that by 1999, perinatal transmission rates declined to 5-7% in a number of areas in Brazil (10, 11, 12, 13, 14, 15). Similar declines have been reported from programs initiated in Argentina (16, 17), Peru (18), Uruguay (19), Bahamas (20), and Trinidad and Tobago (21).

More recently, based on an individual patient meta-analysis and a randomized clinical trial,

cesarean section delivery before labor and ruptured membranes (scheduled cesarean section) has been associated with a substantially decreased risk of vertical transmission of HIV (22, 23). In the absence of antiretroviral therapy, scheduled cesarean section is associated with an approximately 50% decreased risk of transmission of HIV (22, 23). Furthermore, use of zidovudine combined with scheduled cesarean section results in an approximately 87% decreased risk of transmission (22). Although neither study could evaluate the relationship between mode of delivery and mother-to-child transmission according to maternal viral load (22, 23), other studies suggest scheduled cesarean section could be associated with a lower risk of mother-to-child transmission across a range of maternal viral loads (24, 25, 26). Based primarily on the results of the individual patient data meta-analysis and the randomized clinical trial (22, 23), the American College of Obstetricians and Gynecologists now recommends that HIV-infected women with viral loads of greater than 1000 copies/ml should be counseled regarding the potential benefit of cesarean delivery before labor and before rupture of membranes in reducing the risk of vertical transmission of HIV (27). Cesarean section before labor and ruptured membranes for the prevention of vertical transmission is being performed with increasing frequency in the U.S. (28).

Concomitantly, in the U.S., more and more HIV-infected pregnant women have been receiving treatment with potent combination antiretroviral therapy for their own health (29). An estimated 70% of HIV-infected women in the U.S. receive combination antiretroviral therapy during the third trimester of pregnancy, and 35% receive multi-agent therapy including a protease inhibitor (30). Preliminary studies indicate that such treatment may be associated with further reductions in perinatal transmission to rates of 1-2% (31). Similar reductions in transmission among HIV-infected women receiving potent antiretroviral therapy have been reported in Argentina (16).

1.12 Mode of Delivery-Related Morbidity: Maternal and Infant

Maternal

It is well known that, in the absence of HIV infection, cesarean section is associated with increased risks of maternal morbidity. However, sparse data exist regarding a key question facing HIV-infected pregnant women and their clinicians: what is the risk of postpartum morbidity among HIV-infected women with scheduled cesarean section versus other modes of delivery? Among approximately 400 HIV-infected women in the randomized clinical trial (23, 32), postpartum fever was more common among those who delivered via cesarean section. More recently, analyses of approximately 1200 deliveries within the largest North American prospective cohort study of HIV-infected women with postpartum morbidity data (33) revealed scheduled cesarean section was an independent risk factor for postpartum morbidity overall, and for fever without infection, specifically. Among almost 500 HIV-infected women in the PACTG 185 trial, complication rates for women undergoing scheduled cesarean section were intermediate between those for women delivering vaginally and those delivering by urgent cesarean section and were within the range of those reported among similar HIV-negative women (34). Further information is needed regarding the risks associated with scheduled cesarean section to prevent vertical transmission of HIV.

Infant

In general, neonatal morbidity related to scheduled cesarean section would be expected to result from iatrogenic preterm delivery in situations where the gestational age is not accurately assessed prior to delivery. A scheduled cesarean section is generally performed at 39 completed weeks of gestation. However, the American College of Obstetricians and Gynecologists recommends that, for cesarean section undertaken to prevent vertical transmission of HIV, the delivery be performed

at 38 completed weeks of gestation to decrease the chances of ruptured membranes or onset of labor before delivery (27). Even with accurate assessment of gestational age, the relative risk of neonatal respiratory morbidity with delivery by cesarean section before the onset of labor is higher if performed during the 38th week than during the 39th week of gestation (35).

1.13 Antiretroviral Therapy-Related Adverse Events: Maternal and Infant

Maternal

The physiologic changes in pregnancy may increase the frequency or intensity of known toxicities of antiretroviral therapy, impacting compliance as well as maternal and fetal health. For example, anemia is common during pregnancy due to the greater expansion in plasma volume compared to red cell mass, and anemia may be exacerbated by antiretroviral agents that suppress bone marrow production of red blood cells (6). Nausea and vomiting are common during pregnancy and may be worsened by antiretroviral agents. Glucose intolerance may develop in pregnancy because of the anti-insulin effects of human placental lactogen and other hormones, and this tendency could be enhanced by protease inhibitor therapy (36). Recent reports suggest that the risk of severe hepatic dysfunction and lactic acidosis sometimes seen with long term nucleoside antiretroviral therapy may be increased in late pregnancy (37, 38).

Infant

Antiretroviral therapy during pregnancy could increase the rate of pregnancy complications such as preterm birth or fetal growth retardation through direct toxicity, or could improve pregnancy outcome by decreasing viral load and improving maternal health. US and European studies have demonstrated relatively high rates of preterm birth and low birth weight among HIV-infected women, but these rates were similar to those seen in matched control populations of HIV-negative women with similar risk factors for adverse outcomes such as drug abuse and smoking (39, 40, 41). Adverse pregnancy outcome has been associated with maternal disease stage or HIV load in some but not all studies (41, 42, 43, 44). Although early data from the European Collaborative Study suggested maternal antiretroviral therapy was associated with lower rates of preterm birth (45), data from Switzerland as well as more recent data from the European Collaborative Study indicate combination antiretroviral therapy, with or without a protease inhibitor, may be associated with an increased risk of preterm birth (46, 47). Given limited and conflicting data, further study of pregnancy and neonatal outcomes, taking into account maternal treatment and known risk factors for adverse outcomes, is needed.

1.14 Pediatric and Perinatal HIV Clinical Trials

In the United States, the PACTG conducts the vast majority of pediatric and perinatal HIV clinical trials. The PACTG is a collaborative clinical trials group supported by two Institutes at the National Institutes of Health - the National Institute of Child Health and Human Development (NICHD) and the National Institute of Allergy and Infectious Diseases (NIAID). Since 1992, the NICHD has funded a contract research organization, Westat, as the coordinating center for the NICHD Pediatric and Maternal HIV Clinical Trials Network. Westat is responsible for negotiating and managing budgets and subcontracts with clinical trials sites, training of clinical center staff, site monitoring, and data processing, as well as development and management of specific protocols. The NIAID funds the PACTG through a different, cooperative agreement method. Since 1990, investigators supported through these two different mechanisms have worked together in the conduct of pediatric and perinatal clinical trials.

There is increasing international collaboration in clinical trials, both for prevention of perinatal transmission and for treatment of HIV and its complications in children. In recent years the PACTG expanded the perinatal treatment trial, PACTG protocol 316, to international clinical centers. The NICHD supported two sites in Brazil and one in the Bahamas, and the NIAID supported several sites in Europe, for the conduct of this trial. Presently, the PACTG is increasing the international focus and anticipates further collaboration on prevention and treatment trials. The NICHD is working with five Brazilian sites, as well as the Bahamas site, for the performance of a study of enhanced versus regular formula feeding of infants born to HIV-infected women (PACTG 247), to assess whether this might affect the growth and immune function of HIV-infected children at the age of one year. The NIAID recently funded two sites in Thailand and two sites in South Africa for collaboration on future PACTG studies.

The NICHD plans to expand its international collaborations in Latin America and the Caribbean through subcontracts with Westat for the conduct of a multicenter, prospective, observational study of HIV-infected pregnant women and of their HIV-exposed infants. An understanding of the immunologic, virologic and clinical manifestations of HIV infection and exposure in pregnant women and their infants, respectively, is required to assist in the development of an international research agenda. Sites that are chosen to participate in this study must have prior experience in conducting clinical research studies, and the personnel and laboratory capabilities necessary to conduct observational studies. Breast milk alternatives such as formula must be available. Antiretroviral prophylaxis for the prevention of mother-to-child transmission also must be available. NICHD is initiating the current prospective observational study to provide additional information to Latin American and Caribbean countries in which antiretroviral prophylaxis of perinatal transmission is used that might be useful to these countries for the development of future clinical trials.

Intercurrent conditions identified during the course of these protocols that might affect the health or well-being of women and infants in this study will receive appropriate follow-up and care at the clinical centers in which the study is being conducted. Referral to appropriate specialists may occur if the condition is outside the expertise of the investigators.

1.2 Study Rationale

Further information is needed regarding the risks associated with both maternal antiretroviral therapy and scheduled cesarean section to prevent vertical transmission of HIV. Because of the physiologic changes unique to pregnancy and the potential for enhanced toxicity of antiretroviral therapy, data on the frequency and intensity of adverse effects of therapy during pregnancy are required. Data on actual use of agents during pregnancy, including rates of switching for adverse events, are important in developing treatment guidelines and in counseling pregnant women regarding warning signs and symptoms. Given the expanding number of agents available for treatment of HIV, their increasing use during pregnancy, and their unclear impact on pregnancy outcome, further study of pregnancy and neonatal outcomes**,** taking into account maternal treatment and known risk factors for adverse outcomes**,** is imperative. The complications for women and their infants with scheduled cesarean section performed specifically for prevention of HIV transmission compared to other modes of delivery must be described in varied populations to allow better counseling of pregnant women and to identify methods to reduce complications. In addition, the relative benefit of scheduled cesarean section in prevention of HIV transmission among women on various antiretroviral regimens and with a range of HIV RNA levels must be

assessed. Furthermore, this study will explore factors associated with HIV transmission in the few women who transmit despite antiretroviral therapy and/or elective cesarean section.

This study will be a prospective, multicenter, observational study to describe the characteristics of HIV-infected pregnant women and their HIV-exposed infants at participating sites in Latin America and the Caribbean where antiretroviral prophylaxis of mother-to-child transmission of HIV is available. HIV-infected pregnant women receiving medical care at participating clinical sites may be enrolled as early as possible during pregnancy**,** and will be followed for six months postpartum. Their infants will be followed through six months of age, at which point these infants may be enrolled in the NICHD international pediatric protocol.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.11 To describe the characteristics of HIV-infected pregnant women and their HIV-exposed infants receiving care at participating clinical sites in Latin America and the Caribbean, including the utilization of interventions related to decreasing the risk of mother-to-child transmission (antiretroviral therapy, cesarean section before labor and before ruptured membranes, avoidance of breastfeeding); the receipt of antiretroviral or other therapy for the woman's own health; and mother-to-child HIV transmission rates.

2.12 To characterize adverse events during pregnancy and the postpartum period (HIV-infected women) and during early infancy (HIV-exposed infants) according to both mode of delivery and receipt of/exposure to antiretroviral drug(s).

3.0 STUDY DESIGN

This is a prospective cohort study to describe the characteristics of HIV-infected pregnant women and their HIV-exposed infants receiving care at participating clinical sites in Latin America and the Caribbean.

Two groups of patients will be evaluated:
1. HIV-infected pregnant women; and
2. their HIV-exposed infants.

Both groups of patients will have clinical and laboratory evaluations at regular intervals. HIV-infected pregnant women will have prospective data collection during the antepartum and intrapartum periods and for six months postpartum, including history, physical examination, and laboratory evaluations (including hematology, flow cytometry assays, HIV virologic assays, and biochemical assays). HIV-infected pregnant women who experience pregnancy losses also will be followed. In addition, HIV-infected women may be re-enrolled with subsequent pregnancies during this study.

Their HIV-exposed infants will have prospective data collection through six months of age, including history and physical examination, flow cytometry and HIV virologic assays, hematology and biochemical assays, and assessments of growth and morbidity.

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria (HIV-Infected Pregnant Women)

4.11 Documentation of pregnancy, using one or more of the following:
- 4.111 Urine HCG pregnancy test;
- 4.112 Serum HCG pregnancy test;
- 4.113 Positive fetal heart tones by Doppler; or
- 4.114 Ultrasound.

4.12 Documentation of HIV infection, defined by any two of the following prior to or during pregnancy (lab test results must be obtained on separate specimens, i.e. different venipuncture events):
- 4.121 Reactive test for HIV antibody;
- 4.122 Positive HIV culture;
- 4.123 Positive HIV DNA PCR;
- 4.124 Positive neutralizable HIV p24 antigen;
- 4.125 Positive qualitative HIV RNA;
- 4.126 Quantitative HIV RNA ≥ 1000 copies/ml; and
- 4.127 Diagnosis of AIDS-defining clinical condition.

4.13 Willingness and intent to deliver at the participating clinical site and to be followed through six months postpartum at the site or associated outpatient facility

4.14 Willingness and ability to sign informed consent - Subject must be of an age to provide legal informed consent as defined by the country in which the subject resides.

4.15 Willingness and intent to have infant followed through six months of age

4.16 Subjects may be co-enrolled in clinical trials for treatment or prophylaxis of HIV infection, opportunistic infections, or other HIV-related problems.

4.17 Subjects may be re-enrolled with subsequent pregnancies during this study.

4.18 Subjects may enroll up to and prior to delivery, including during labor.

4.2 Exclusion Criteria (HIV-Infected Pregnant Women): Failure to meet inclusion criteria

4.3 Enrollment Procedures

Prior to the collection of any data, patients will be provided ample time to review the informed consent document, have questions answered, and discuss any concerns related to study participation. Patients will be encouraged to enroll at the earliest possible prenatal visit.

5.0 CLINICAL AND LABORATORY EVALUATIONS

Women will be encouraged to enroll as early as possible in pregnancy. The acceptable window for the six-month postpartum visit for the mother and the six-month postnatal visit for the infant is +/- two months. Missed visits should be rescheduled as soon as possible.

Enrollment at ≤ 16 weeks gestation

If the woman is enrolled at or before 16 weeks gestation, then the study visit schedule will be as follows:

1. First antepartum (enrollment) visit at 8-16 weeks gestation;

2. Second antepartum visit at 20-28 weeks gestation;
3. Third antepartum visit at 32-36 weeks gestation;
4. Delivery, or other pregnancy outcome**;**
5. Prior to hospital discharge after delivery, or after other pregnancy outcome;
6. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
7. Postpartum visit at six months after delivery, or after other pregnancy outcome.

Enrollment after 16 weeks but ≤ 28 weeks gestation

If the woman is not enrolled by 16 weeks gestation, but can be enrolled at or before 28 weeks gestation, then the study visit schedule will be as follows:

1. First antepartum (enrollment) visit at 20-28 weeks gestation;
2. Second antepartum visit at 32-36 weeks gestation;
3. Delivery, or other pregnancy outcome;
4. Prior to hospital discharge after delivery, or after other pregnancy outcome;
5. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
6. Postpartum visit at six months after delivery, or after other pregnancy outcome.

Enrollment after 28 weeks but ≤ 36 weeks gestation

If the woman is not enrolled by 28 weeks gestation, but can be enrolled at or before 36 weeks gestation, then the study visit schedule will be as follows:

1. First antepartum (enrollment) visit at 32-36 weeks gestation;
2. Delivery, or other pregnancy outcome;
3. Prior to hospital discharge after delivery, or after other pregnancy outcome;
4. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
5. Postpartum visit at six months after delivery, or after other pregnancy outcome.

Enrollment after 36 weeks but before onset of labor or other pregnancy outcome

If the woman is enrolled after 36 weeks gestation but before onset of labor, or other pregnancy outcome, then the study visit schedule will be as follows:

1. First antepartum (enrollment) visit upon study entry for those women who enroll before onset of labor;
2. Delivery, or other pregnancy outcome;
3. Prior to hospital discharge after delivery, or after other pregnancy outcome;
4. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
5. Postpartum visit at six months after delivery, or after other pregnancy outcome.

Enrollment after onset of labor

If the woman is enrolled after the onset of labor, then the study visit schedule will be as follows:

1. Delivery;
2. Prior to hospital discharge after delivery, or after other pregnancy outcome;
3. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
4. Postpartum visit at six months after delivery, or after other pregnancy outcome.

Infant Enrollment

The study visit schedule for the infants will mirror that of the women after delivery:

1. Prior to hospital discharge after birth;
2. At 6-12 weeks of age; and
3. At six months of age.

Specimen Storage for Women and Infants

Plasma and peripheral blood mononuclear cell (PBMC) samples for storage will be obtained from women at the first, second and third antepartum visits, prior to hospital discharge, at 6-12 weeks postpartum, and at six months postpartum. Plasma and PBMC samples for storage will be obtained from infants prior to hospital discharge, at 6-12 weeks after delivery, and at six months after delivery.

- Samples will be stored for an indefinite period of time at a central repository and identified by a unique patient identification number (PID). Samples will only be used for studies relating to HIV infection and its complications.
- The research studies that might be performed on stored blood specimens could include studies to understand how HIV causes disease, and how to best treat or prevent HIV infection and its complications. Specific studies might include new ways of measuring the amount of HIV in the blood; measures of viral resistance and factors that might affect resistance; measures of antiretroviral drug concentrations and biological factors that might affect concentrations; and new measures of toxicity. Patients may choose not to participate in this portion of the protocol or they may choose to withdraw their specimens at any time. Results of these studies, although experimental, will be provided to the study site physicians.

NOTE: See Appendix V for details regarding collection, processing, storage and transportation of specimens.

5.1 HIV-Infected Women

5.11 First antepartum visit (enrollment visit)

5.111 History (or interval history since most recent visit)

- Demographic data
- HIV testing
- General medical history
- Reproductive history
- Prenatal history
- Antiretroviral therapy history
- Other medication history

5.112 Intended mode of delivery (trial of labor versus scheduled cesarean section)

5.113 Physical examination

5.114 Laboratory evaluations

- Peripheral blood viral load (HIV RNA)
- Complete blood count with differential and platelets
- Flow cytometry (CD4+ and CD8+ absolute counts and percents)
- Biochemical assays (See Appendix I-A.)
- Specimens for storage: plasma and peripheral blood mononuclear cells (PBMCs)

5.12 Second antepartum visit

5.121 History

- General medical history
- Antiretroviral therapy history
- Other medication history
- Other history, if not obtained previously (demographic data, HIV testing, reproductive history, prenatal history)

5.122 Intended mode of delivery
5.123 Physical examination, signs/symptoms, diagnoses
5.124 Laboratory evaluations

- Peripheral blood viral load (HIV RNA)
- Complete blood count with differential and platelets
- Flow cytometry (CD4+ and CD8+ absolute counts and percents)
- Biochemical assays (See Appendix I-A.)
- Specimens for storage: plasma and peripheral blood mononuclear cells (PBMCs)

5.13 Third antepartum visit

5.131 History

- General medical history
- Antiretroviral therapy history
- Other medication history
- Other history, if not obtained previously (demographic data, HIV testing, reproductive history, prenatal history)
- Medical record abstraction of selected laboratory results (if available) (See Appendix I-A.)

5.132 Intended mode of delivery
5.133 Physical examination, signs/symptoms, diagnoses
5.134 Laboratory evaluations

- Peripheral blood viral load (HIV RNA)
- Complete blood count with differential and platelets
- Flow cytometry (CD4+ and CD8+ absolute counts and percents)
- Biochemical assays (See Appendix I-A.)
- Vaginal swab (slide for Gram stain), to be done at third antepartum visit, or if seen for less than three antepartum visits, to be done after 32 weeks gestation
- Specimens for storage: plasma and peripheral blood mononuclear cells (PBMCs)

5.14 Labor and Delivery

5.141 History

- General medical history
- Antiretroviral therapy history
- Other medication history
- Other history, if not obtained previously (demographic data, HIV testing, reproductive history, prenatal history)

5.142 Delivery history

5.15 Prior to hospital discharge

5.151 Interval history

- General medical history
- Antiretroviral therapy history
- Other medication history
- If not done previously, medical record abstraction of selected laboratory results (if

available) (See Appendix I-A.)

5.152 Physical examination, signs/symptoms, diagnoses (including postpartum morbidity; see Appendix I-A.)

5.153 Laboratory evaluations

- Peripheral blood viral load (HIV RNA)
- Complete blood count with differential and platelets
- Flow cytometry (CD4+ and CD8+ absolute counts and percents)
- Specimens for storage: plasma and peripheral blood mononuclear cells (PBMCs)

5.16 Postpartum visit at 6-12 weeks after delivery or other pregnancy outcome

5.161 Interval history

- General medical history
- Antiretroviral therapy history
- Other medication history

5.162 Physical examination, signs/symptoms, diagnoses (including postpartum morbidity; see Appendix I-A.)

5.163 Laboratory evaluations

- Peripheral blood viral load (HIV RNA)
- Complete blood count with differential and platelets
- Flow cytometry (CD4+ and CD8+ absolute counts and percents)
- Biochemical assays (See Appendix I-A.)
- Specimens for storage: plasma and peripheral blood mononuclear cells (PBMCs)

5.17 Postpartum visit at six months after delivery or other pregnancy outcome

5.171 Interval History

- General medical history
- Antiretroviral therapy history
- Other medication history

5.2 HIV-Exposed Infants

5.21 Prior to hospital discharge, at 6-12 weeks of age, and at six months of age

5.211 Birth history or interval history since most recent visit (including feeding history)

5.212 Physical examination (including weight, height and head circumference), signs/symptoms, diagnoses (to include respiratory and infectious morbidity in particular)

5.213 Laboratory evaluations

- Diagnostic assay for HIV infection (See list in Appendix II.)
- Complete blood count with differential and platelets
- Flow cytometry (CD4+ and CD8+ absolute counts and percent)
- AST (SGOT); ALT (SGPT)
- Specimens for storage: plasma and peripheral blood mononuclear cells (PBMCs)

6.0 STATISTICAL CONSIDERATIONS

6.1 Design Considerations

This non-randomized, observational study will prospectively collect history, physical examination, and laboratory data from HIV-infected pregnant women and their HIV-

exposed infants. HIV-infected pregnant women receiving medical care at participating clinical sites that plan to deliver at the clinical site, and plan to continue postpartum medical care for six months at the clinical site or associated outpatient facility, will be eligible. Data will be collected for HIV-infected women during the antepartum, intrapartum, and six months postpartum periods. Data will be collected for HIV-exposed infants before hospital discharge after birth, at 6-12 weeks of age, and at ***six*** months of age. Data collection regarding HIV-infected women will entail history, physical examination and laboratory evaluations (including hematology, flow cytometry, HIV virologic assays, and biochemical assays). Data collection regarding HIV-exposed infants also will include history, physical exam, and laboratory evaluations.

The first objective involves describing the characteristics of HIV-infected pregnant women and their HIV-exposed infants receiving care at participating clinical sites. In addition, the utilization of interventions related to decreasing the risk of mother-to-child transmission (antiretroviral therapy, cesarean section before labor and before ruptured membranes, avoidance of breastfeeding), the receipt of antiretroviral or other therapy for the woman's own health, and mother-to-child HIV transmission rates will be described. Secondly, maternal and infant adverse events, e.g., anemia, nausea and vomiting, preterm birth and fetal growth retardation will be characterized both by mode of delivery and receipt of or exposure to antiretroviral drug(s).

Participating clinical sites are asked to enroll all eligible patients into the study and to provide their usual therapeutic standard of care; this study does not include randomization or stratification, and the study does not include provision of study medications because it is an observational study. Sites must have available breast milk alternatives such as formula. They must have available antiretroviral prophylaxis for the prevention of mother-to-child transmission of HIV. This study is designed primarily to undertake descriptive analyses and not for testing formal hypotheses. Comparisons between subgroups of patients may be undertaken whenever sufficient sample size and adequate statistical power are available.

6.2 Sample Size and Accrual

Based on a minimum of 30 HIV-infected pregnant women enrolled at each site per year from a minimum of six to eight participating sites, it is anticipated that between 180 to 240 women would be enrolled during a one-year study. If additional clinical sites participate or the study expands to two to five years, then estimates of prevalence (e.g., prevalence of fetal growth retardation) will be more precise.

The sample size will be evaluated by 95% confidence interval (C.I.) widths of prevalences. The 95% C.I. associated with the estimated prevalence of a given adverse event (e.g., anemia) is a function of the true prevalence, P_V, and the number of subjects evaluated for the characteristic. The exact 95% C.I. for a given sample size and prevalence, P_V, is based on the relationship between the binomial and $F-$distribution (48).

The following table[1] displays the 95% C.I.s for estimated prevalences between 1% and 50% based on the number of subjects being between 200 and 1000. For example, assuming a given prevalence outcome of 10%, then the exact 95% C.I. width equals 5.5% (13%-7.5%=5.5%) with a sample of 500 subjects.

n

P_v (%)	**200**	**300**	**400**	**500**	**600**	**700**	**800**	**900**	**1000**
1	(0.1- 3.6)	(0.2- 2.9)	(0.3- 2.5)	(0.3- 2.3)	(0.4- 2.2)	(0.4- 2.0)	(0.4- 2.0)	(0.5- 1.9)	(0.5- 1.8)
2	(0.5- 5.0)	(0.7- 4.3)	(0.9- 3.9)	(1.0- 3.6)	(1.0- 3.5)	(1.1- 3.3)	(1.1- 3.2)	(1.2- 3.1)	(1.2- 3.1)
3	(1.1- 6.4)	(1.4- 5.6)	(1.6- 5.2)	(1.7- 4.9)	(1.8- 4.7)	(1.9- 4.5)	(1.9- 4.4)	(2.0- 4.3)	(2.0- 4.3)
4	(1.7- 7.7)	(2.1- 6.9)	(2.3- 6.4)	(2.5- 6.1)	(2.6- 5.9)	(2.7- 5.7)	(2.8- 5.6)	(2.8- 5.5)	(2.9- 5.4)
5	(2.4- 9.0)	(2.8- 8.1)	(3.1- 7.6)	(3.3- 7.3)	(3.4- 7.1)	(3.5- 6.9)	(3.6- 6.7)	(3.7- 6.6)	(3.7- 6.5)
10	(6.2-15.0)	(6.8-14.0)	(7.2-13.4)	(7.5-13.0)	(7.7-12.7)	(7.9-12.5)	(8.0-12.3)	(8.1-12.1)	(8.2-12.0)
15	(10.4-20.7)	(11.2-19.6)	(11.6-18.9)	(12.0-18.4)	(12.2-18.1)	(12.4-17.9)	(12.6-17.7)	(12.7-17.5)	(12.8-17.4)
20	(14.7-26.2)	(15.6-25.0)	(16.2-24.3)	(16.6-23.8)	(16.9-23.4)	(17.1-23.2)	(17.3-22.9)	(17.4-22.8)	(17.6-22.6)
30	(23.7-36.9)	(24.9-35.5)	(25.5-34.8)	(26.0-34.2)	(26.4-33.8)	(26.6-33.5)	(26.8-33.3)	(27.0-33.1)	(27.2-32.9)
40	(33.2-47.1)	(34.4-45.8)	(35.2-45.0)	(35.7-44.4)	(36.1-44.0)	(36.3-43.7)	(36.6-43.5)	(36.8-43.3)	(36.9-43.1)
50	(42.9-57.1)	(44.2-55.8)	(45.0-55.0)	(45.5-54.5)	(45.9-54.1)	(46.2-53.8)	(46.5-53.5)	(46.7-53.3)	(46.9-53.1)

Sample size calculations may also be based on the lower bound of the 95% C.I. for the true prevalence. One can calculate the power to conclude that the upper (lower) bound of an exact one-sided C.I. (49) for the true prevalence does not (does) exceed a certain value. For example, 500 subjects would provide approximately 94% power (coverage probability 96%) to conclude that the lower limit of the exact one-sided 95% C.I. exceeds 6% given that the true prevalence is 10%. Alternatively, one would have 83% power (coverage probability 96%) to conclude that the upper limit of the exact one-sided 95% C.I. does not exceed 14% given a true prevalence of 10%.

[1] Shaded entries correspond to confidence interval widths narrower than 10%, e.g., if the prevalence is 10% or less then sample sizes of 200 or higher yield a 95% confidence interval widths narrower than 10%.

6.3 Analysis Plans

Most analyses (for HIV-infected pregnant women and their infants) will focus on estimating the prevalence (and 95% C.I.s) of clinical outcomes. Frequency distribution of test results for continuous measures (e.g., viral load, CD4+ counts) and standard summary statistics (e.g. mean, median, standard error, range) will also be reported, by mode of delivery and receipt of or exposure to antiretroviral drug(s) where appropriate. The occurrence of major and minor postpartum morbidity (e.g., endometritis and urinary tract infection) and of neonatal morbidity (e.g., respiratory distress syndrome) will be analyzed according to actual mode of delivery (spontaneous vaginal delivery, vaginal delivery with forceps and/or vacuum, scheduled cesarean section, and other [non-elective] cesarean section). The occurrence of adverse events such as low birth weight and preterm birth will be analyzed according to receipt of antiretroviral drugs during pregnancy.

One limitation of the analyses may be the small sample sizes that are likely to exist, especially for subgroup analyses. The sample sizes, however, are sufficient to obtain reasonably precise estimates of prevalence and clinical test results, which could be used to address the objectives of the study, and for group comparisons that do not involve rare outcomes.

A second limitation relates to the nature of observational studies. Biased results may be obtained in the comparison of the safety and efficacy of treatments in observational studies, because patients were not randomized to study treatments. Techniques such as propensity score models (50, 51) that attempt to control for this type of bias could be considered.

6.4 Monitoring and Interim Analyses

Accrual of HIV-infected pregnant women will be monitored and compiled at the individual site level as well as all sites combined. Accrual will be reported on a monthly basis, showing the history of accrual for each month and the cumulative total accrual. Follow-up visit rates for HIV-infected mothers and their infants will be monitored and reported. The percentage of subjects lost to follow-up also will be calculated.

7.0 DATA COLLECTION AND STUDY MONITORING

7.1 Data Entry and Collection

Study data will be recorded on Case Report Forms (CRFs) that will be provided to study sites for each participating subject by Westat. Subjects must not be identified by name on any study documents. Subjects will be identified by the Patient Identification Number (PID) provided by Westat upon site registration for the protocol.

All data on the CRF must be legibly recorded in black ink or typed. 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. Any requested information that is not obtained as specified in the protocol should have an explanation noted in the source documentation as to why the required information was not obtained.

Copies of the CRFs must be kept on file at each clinical center, and all records prepared during this study must be retained until otherwise authorized by the NICHD.

7.2 Regional Monitoring

Site monitors contracted with the National Institute of Child Health and Human Development (NICHD) will visit participating clinical sites on a regular basis to review medical records of research participants, regulatory documents, case report forms (CRFs), and any other documents prepared during the conduct of this study. Site monitors will review records for accuracy, completeness, and legibility, and will inspect regulatory files to ensure that all regulatory requirements are being followed. The investigator will make study documents (e.g., consent forms, case report forms) and pertinent hospital or clinic records readily available for inspection by the site monitor(s) for confirmation of the study data.

7.3 Site Performance Reports

Monthly reports will be sent to the sites summarizing their patient accrual and follow-up visit progress for the measures describe above. Summary reports also will be issued that indicate data submission activities, including number of forms submitted and number of data queries pending.

8.0 HUMAN SUBJECTS PROTECTION

8.1 Racial/Ethnic, Gender and Age Statement

Members of all racial and ethnic groups will be eligible for recruitment into this study. As this is a prospective, observational study of outcomes potentially related to antepartum/in utero exposure to antiretroviral medications and to mode of delivery, only HIV-infected pregnant women will be enrolled. Strategies for recruitment will predominantly involve identifying HIV-infected pregnant women at prenatal visits through counseling and testing programs at clinical sites.

8.2 Evaluation of Benefits and Risks/Discomforts

Women who enter this protocol will be HIV-infected. Many women may have just learned of their diagnosis through testing programs offered to them during pregnancy. As part of routine care women will be counseled regarding treatment options as well as interventions to decrease maternal-infant transmission, including antiretroviral prophylaxis, cesarean section before labor and ruptured membranes, and avoidance of breastfeeding. As a prospective, observational cohort study, the risk to the subject is likely to be no more than minimal risk. There is a small risk of bruising or discomfort at the site of venipuncture. Tests and physical exams to be performed through this study are primarily those that would occur for routine care of this patient population had they not been enrolled in a study, except for an additional visit at six months postpartum. This

visit is to assess potential late delivery complications in the mother and to evaluate toxicity in her infant. Although subjects will not be compensated for their participation, clinical sites may provide incentive payments in the form of transportation to the site or meals. Benefits to the subject include the repeated HIV testing for diagnostic purposes of her infant. Infants of mothers who enter this protocol will have been exposed in utero to human immunodeficiency virus and/or antiretroviral medications used for its treatment and prevention. Short-term outcomes potentially related to this exposure will be assessed in these infants.

8.3 Ethics Committee Review and Informed Consent

All participating centers must be in compliance with U.S. and in-country local/national regulations applicable to research involving human subjects, in accordance with the International Conference on Harmonisation (ICH)/Good Clinical Practices (GCP) guidelines. Should U.S. and in-country local/national regulations differ, the more restrictive guidelines will apply.

This is an observational, prospective cohort study; all treatment received by individuals enrolled in this observational study is prescribed by clinicians caring for the enrolled individuals independent of this protocol. Since no protocol-mandated intervention is being administered as part of this protocol, adverse events will not be reported. Since this is an observational protocol of no greater than minimal risk, DSMB review is not required.

This protocol, the informed consent document (Appendix VI), and any subsequent modifications will be reviewed and approved by the Ethics Committee or Institutional Review Board (IRB) responsible for oversight of the study, including any national Ethics Committee or IRB. Written informed consent will be obtained from the subject. The mother must give written informed consent for her baby's participation in the study. The informed consent 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 subject. Subjects who are unable to read or write should undergo the same informed consent process as literate subjects; however, the informed consent forms will be read to them. In lieu of a signature, the subject's thumbprint will be taken by pressing the thumb onto a regular inkpad and pressing the imprint onto the appropriate line of the consent. In addition to the subject's signature (or thumb print) and date, the staff person conducting the consent discussion must sign the informed consent form. The informed consent process must be witnessed by a third individual who is required to sign the consent form as a witness.

8.4 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified by a coded number only, to maintain subject confidentiality. All records will be kept in a secured area. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, parent, or guardian, except as necessary for monitoring by the NICHD, the local Ethics Committee or IRB, and/or the country's national Ethics Committee, IRB, or Ministry of Health**.**

8.5 Study Discontinuation

The local Ethics Committee or IRB, the country's national Ethics Committee, the country's Ministry of Health, or the NICHD may discontinue this study at any time.

8.6 Required Education in the Protection of Human Research Participants

NIH policy requires education on the protection of human subject participants for all investigators receiving NIH contract and subcontract awards for research involving human subjects. For a complete description of the NIH Announcement on required education in the protection of human subject participants, the subcontractor should access the NIH Guide for Grants and Contracts Announcement dated June 5, 2000 at the following website:
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-00-039.html.

9.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this study will be governed by the protocol team. Any presentation, abstract, or manuscript will be made available for review by the protocol team 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 al specimens for this study, as currently recommended by the Centers for Disease Control and Prevention.

All infectious specimens will be sent using the ISS-1 SAF-T-PAK mandated by the international Air Transport Association Dangerous Goods Regulations-Packing Instruction 602. Please refer to individual carrier guidelines (e.g., FedEx, Airborne, World Courier) for specific instructions.

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APPENDIX I-A

SCHEDULE OF EVALUATIONS: **HIV-INFECTED WOMEN**

Evaluation	1st Antepartum Visit: Enrollment[1]	2nd Antepartum Visit	3rd Antepartum Visit	Labor & Delivery	Prior to Hospital Discharge	6-12 Weeks Postpartum	Six Months Postpartum (+/- 2 mths.)
Informed Consent	X						
Documentation of Mother's HIV Infection Status & Pregnancy	X						
Intended Mode of Delivery	X	X	X				
HISTORY							
History	X						
Medical Record Abstraction of Selected Laboratory Results (if available)[2]			X		X* *if not done at 3rd antepartum visit		
Interval History		X	X	X	X	X	X
PHYSICAL EXAMINATION							
Physical Exam	X						
Physical Exam, Signs/Symptoms, Diagnoses		X	X	X	X[3]	X[3]	
LABORATORY EVALUATIONS (in highest to lowest priority order)							
Complete Blood Count with Differential and Platelets	X (EDTA purple) (2.0 ml)	X (2.0 ml)	X (2.0 ml)		X (2.0 ml)	X (2.0 ml)	
Biochemical Assays[4]	X (B. D. red) (2.0 ml)	X (2.0 ml)	X (2.0 ml)			X (2.0 ml)	
Peripheral Blood Viral Load (HIV RNA)	X (EDTA purple) (2.0 ml)	X (2.0 ml)	X (2.0 ml)		X (2.0 ml)	X (2.0 ml)	
Flow Cytometry (CD4+ and CD8+ absolute counts and percentages)	X (EDTA purple) (2.0 ml)	X (2.0 ml)	X (2.0 ml)		X (2.0 ml)	X (2.0 ml)	
Specimens for Storage: plasma and peripheral blood mononuclear cells (PBMCs)[6]	X (ACD yellow) (6.0 ml)	X (6.0 ml)	X (6.0 ml)		X (6.0 ml)	X (6.0 ml)	
Vaginal Swab (slide for Gram stain)			X[5]				
Maximum Total Blood Volume	14 ml	14 ml	14 ml		12 ml	14 ml	

FOOTNOTES TO APPENDIX I-A - SCHEDULE OF EVALUATIONS: HIV-INFECTED WOMEN

[1] Enrollment will occur at the first available antepartum visit during any trimester, and women will be encouraged to enroll as early as possible in pregnancy so that as many as possible of the scheduled antepartum visits are completed, as described below.

Enrollment at ≤ 16 weeks gestation

If the woman is enrolled at or before 16 weeks gestation, then the study visit schedule will be as follows:

1. First antepartum (enrollment) visit at 8-16 weeks gestation;
2. Second antepartum visit at 20-28 weeks gestation;
3. Third antepartum visit at 32-36 weeks gestation;
4. Delivery, or other pregnancy outcome**;**
5. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
6. Postpartum visit at six months after delivery, or after other pregnancy outcome.

Enrollment after 16 weeks but ≤ 28 weeks gestation

If the woman is not enrolled by 16 weeks gestation, but can be enrolled at or before 28 weeks gestation, then the study visit schedule will be as follows:

1. First antepartum (enrollment) visit at 20-28 weeks gestation;
2. Second antepartum visit at 32-36 weeks gestation;
3. Delivery, or other pregnancy outcome;
4. Prior to hospital discharge after delivery, or after other pregnancy outcome;
5. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
6. Postpartum visit at six months after delivery, or after other pregnancy outcome.

Enrollment after 28 weeks but ≤ 36 weeks gestation

If the woman is not enrolled by 28 weeks gestation, but can be enrolled at or before 36 weeks gestation, then the study visit schedule will be as follows:

1. First antepartum (enrollment) visit at 32-36 weeks gestation;
2. Delivery, or other pregnancy outcome;
3. Prior to hospital discharge after delivery, or after other pregnancy outcome;
4. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
5. Postpartum visit at six months after delivery, or after other pregnancy outcome.

FOOTNOTES TO APPENDIX I-A - SCHEDULE OF EVALUATIONS: HIV-INFECTED WOMEN (continued)

Enrollment after 36 weeks but before onset of labor, or other pregnancy outcome

If the woman is enrolled after 36 weeks gestation but before onset of labor, or other pregnancy outcome, then the study visit schedule will be as follows:

1. First antepartum (enrollment) visit upon study entry for those women who enroll before onset of labor;
2. Delivery, or other pregnancy outcome;
3. Prior to hospital discharge after delivery, or after other pregnancy outcome;
4. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
5. Postpartum visit at six months after delivery, or after other pregnancy outcome.

Enrollment after onset of labor

If the woman is enrolled after the onset of labor, then the study visit schedule will be as follows:

1. Delivery;
2. Prior to hospital discharge after delivery, or after other pregnancy outcome;
3. Postpartum visit at 6-12 weeks after delivery, or after other pregnancy outcome; and
4. Postpartum visit at six months after delivery, or after other pregnancy outcome.

[2] Medical record abstraction of selected laboratory tests results, if available; for example: blood type, Rh, antibody screen, urinalysis and urine culture, syphilis serology, hepatitis B profile, hepatitis C antibody and hepatitis C RNA PCR if antibody positive, rubella, varicella, HSV type specific antibody, toxoplasma, CMV antibody; results of pelvic examination(s); maternal serum screening results (AFP); glucose tolerance testing

[3] Postpartum morbidity evaluation diagnoses: Major and minor morbidity to include the following: febrile morbidity, infections(including endometritis, urinary tract infections (cystitis and pyelonephritis), wound complications, pneumonia, peritonitis, sepsis), anemia, hemorrhage (including those requiring surgical procedures and/or transfusion), pleural effusion, thromboembolic events, and disseminated intravascular coagulation.

[4] Biochemical assays to include: liver and pancreatic function tests [AST (SGOT), ALT (SGPT), bilirubin, albumin, total protein, amylase], renal function (blood urea nitrogen (BUN), creatinine), blood chemistries (sodium, potassium, chloride, bicarbonate), and other assays (blood glucose, cholesterol, triglycerides).

[5] Vaginal swab to be done at third antepartum visit, or if seen for less than three antepartum visits, to be done after 32 weeks gestation.

[6] Refer to Appendix V for guidelines regarding collection, processing, and transportation of these specimens.

APPENDIX I-B
SCHEDULE OF EVALUATIONS: **HIV-EXPOSED INFANTS**

Evaluation	Prior To Hospital Discharge	6-12 Weeks of Age	Six Months of Age (+/- 2 months)
HISTORY			
Birth History	X		
Interval History		X	X
PHYSICAL EXAMINATION			
Physical Examination (including weight, height and head circumference), Signs/Symptoms, Diagnoses[1]	X	X	X
LABORATORY EVALUATIONS (in highest to lowest priority order)			
Diagnostic test for HIV (See list in Appendix II.)	X (2.0 ml) (EDTA purple)	X (2.0 ml)	X (2.0 ml)
Complete Blood Count with Differential and Platelets[4]	X (EDTA purple) (2.0 ml)	X (2.0 ml)	X (2.0 ml)
AST (SGOT); ALT (SGPT)[3]	X (B.D. red -no additive) (2.0 ml)	X (2.0 ml)	X (2.0 ml)
Flow Cytometry (CD4+ and CD8+ absolute counts and percentages)[4]	X (with CBC)	X (with CBC)	X (with CBC)
Specimens for Storage: plasma and peripheral blood mononuclear cells (PBMCs)[2]	X (EDTA purple) (2.0 ml)	X (2.0 ml)	X (2.0 ml)
Maximum Total Blood Volume	8.0 ml	8.0 ml	8.0 ml

[1] Diagnoses to include respiratory and infectious morbidity in particular.
[2] Refer to Appendix V for guidelines regarding collection, processing, and transportation of these specimens. Use Becton Dickinson tube No. 369651 or equivalent.
[3] A further reduction in required blood volume may be achieved with the use of a Becton Dickinson Microtainer red top tube without additives, (No. 365957) with a volume of 800-900 uL.
[4] Collection of blood for flow cytometry will be with the same 2.0 ml EDTA purple tube as the complete blood count with differential and platelets. Use Becton Dickinson tube No. 369651 or equivalent.

NOTE: The maximum volume at each infant blood draw is 3 ml/kg of body weight.

APPENDIX II

DEFINITION OF INFANT HIV INFECTION STATUS

The protocol will utilize the following definitions of HIV infection status for the purpose of determining infant infection status.

A. HIV-infected

Any two of the following test results on separate specimens (different venipuncture events):

- Positive HIV culture;
- Positive HIV DNA PCR;
- Positive neutralizable HIV p24 antigen;
- Quantitative HIV RNA ≥ 1,000 copies/ml.

B. HIV-uninfected

- Two or more negative HIV virologic assays (e.g., HIV culture or HIV DNA PCR) with one test performed at age one month or older and one performed at age four months or older, and no positive virologic tests.

Or

- One positive HIV virologic assay with at least two later negative HIV virologic tests, at least one of which is after age four months or negative HIV antibody test results at least one of which is after age six months.

C. HIV-indeterminate

- Meets neither of the above definitions.

APPENDIX III
ADULT HIV CLASSIFICATION

1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults

Summary

CDC has revised the classification system for HIV infection to emphasize the clinical importance of the CD4+ T-lymphocyte count in the categorization of HIV-related clinical conditions. This classification system replaces the system published by CDC in 1986 (1) and is primarily intended for use in public health practice. Consistent with the 1993 revised classification system, CDC has also expanded the AIDS surveillance case definition to include all HIV-infected persons who have less than 200 CD4+ T-lymphocytes/uL, or a CD4+ T-lymphocyte percentage of total lymphocytes of less than 14. This expansion includes the addition of three clinical conditions

- pulmonary tuberculosis, recurrent pneumonia, and invasive cervical cancer -- and retains the 23 clinical conditions in the AIDS surveillance case definition published in 1987 (2); it is to be used by all states for AIDS case reporting effective January 1, 1993.

CD4+ T-Lymphocyte Categories

The three CD4+ T-lymphocyte categories are defined as follows:

- Category 1: greater than or equal to 500 cells/uL
- Category 2: 200-499 cells/uL
- Category 3: less than 200 cells/uL

These categories correspond to CD4+ T-lymphocyte counts per microliter of blood and guide clinical and therapeutic actions in the management of HIV-infected adolescents and adults (22-28). The revised HIV classification system also allows for the use of the percentage of CD4+ T-cells (Appendix A).

HIV-infected persons should be classified based on existing guidelines for the medical management of HIV-infected persons (22). Thus, the lowest accurate, but not necessarily the most recent, CD4+ T-lymphocyte count should be used for classification purposes.

Clinical Categories

The clinical categories of HIV infection are defined as follows:

Category A

Category A consists of one or more of the conditions listed below in an adolescent or adult (greater than or equal to 13 years) with documented HIV infection. Conditions listed in Categories B and C must not have occurred.

- Asymptomatic HIV infection
- Persistent generalized lymphadenopathy
- Acute (primary) HIV infection with accompanying illness or history of acute HIV infection (29,30)

Category B

Category B consists of symptomatic conditions in an HIV-infected adolescent or adult that are not included among conditions listed in clinical Category C and that meet at least one of the following criteria: a) the conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or b) the conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection. Examples of conditions in clinical Category B include, but are not limited to:

- Bacillary angiomatosis
- Candidiasis, oropharyngeal (thrush)
- Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy
- Cervical dysplasia (moderate or severe)/cervical carcinoma in situ
- Constitutional symptoms, such as fever (38.5 C) or diarrhea lasting greater than 1 month
- Hairy leukoplakia, oral
- Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome
- Idiopathic thrombocytopenic purpura
- Listeriosis
- Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess
- Peripheral neuropathy

For classification purposes, Category B conditions take precedence over those in Category A. For example, someone previously treated for oral or persistent vaginal candidiasis (and who has not developed a Category C disease) but who is now asymptomatic should be classified in clinical Category B.

Category C

Category C includes the clinical conditions listed in the AIDS surveillance case definition (Appendix B). For classification purposes, once a Category C condition has occurred, the person will remain in Category C.

APPENDIX B. Conditions included in the 1993 AIDS surveillance case definition

- Candidiasis of bronchi, trachea, or lungs
- Candidiasis, esophageal
- Cervical cancer, invasive *
- Coccidioidomycosis, disseminated or extrapulmonary
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic intestinal (greater than 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy, HIV-related
- Herpes simplex: chronic ulcer(s) (greater than 1 month's duration); or bronchitis, pneumonitis, or esophagitis
- Histoplasmosis, disseminated or extrapulmonary
- Isosporiasis, chronic intestinal (greater than 1 month's duration)
- Kaposi's sarcoma
- Lymphoma, Burkitt's (or equivalent term)

- Lymphoma, immunoblastic (or equivalent term)
- Lymphoma, primary, of brain
- Mycobacterium avium complex or M. kansasii, disseminated or extrapulmonary
- Mycobacterium tuberculosis, any site (pulmonary * or extrapulmonary)
- Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
- Pneumocystis carinii pneumonia
- Pneumonia, recurrent *
- Progressive multifocal leukoencephalopathy
- Salmonella septicemia, recurrent
- Toxoplasmosis of brain
- Wasting syndrome due to HIV
- Added in the 1993 expansion of the AIDS surveillance case definition.

NOTES:

1) This system will be utilized by the protocol to provide a standardized classification system. This system is not intended to provide clinical care standards.
2) Please see Morbidity and Mortality Weekly Report (MMWR), 41(RR-17), December 18, 1992 for full text, references and acknowledgments.

APPENDIX IV

WEIGHT AND GROWTH MEASUREMENTS

How to Measure Head Circumference:

The tape is placed across the forehead with the lower border of the tape just above the eyebrows, around the head, above the ears and over the occipital prominence at the back of the head. Pull tape firmly to compress the hair and underlying soft tissues. Positioning of the tape over the forehead and occiput should be done to yield the maximum head circumference. Record head circumference in centimeters to the nearest 0.1 cm.

How to Measure Length:

Measure supine on a standard measuring board. Keep infant's legs straight and toes up, bringing moveable footboard to rest firmly against child's heels. Measure length in centimeters to the nearest 0.1 cm.

How to Measure Weight:

All weights should be obtained without diapers and clothing; whenever possible, the same scales should be used for these measurements.

APPENDIX V

SPECIMEN COLLECTION, PROCESSING, STORAGE, AND TRANSPORTATION PROCEDURES

The purpose of this appendix is to describe specimen collection, processing, storage, and transportation procedures regarding repository specimens (plasma and peripheral blood mononuclear cells (PBMCs) obtained for future analyses).

Laboratory practices should be employed to promote a safe workplace and to prevent contamination of specimens. Specific requirements include:

- Perform work in an area that does not communicate with any laboratory airspace, equipment, personnel or reagents that are involved in post PCR-amplification processes.
- Use aseptic techniques.
- Use a biological safety hood, under which all procedures will be performed.

Further details regarding laboratory procedures are available online in the DAIDS Virology Manual for HIV Laboratories at http://www.niaid.nih.gov/daids/vir_manual/ and the ACTG Immunology Consensus Methods webpage at http://aactg.s-3.co/immmeth.htm.

BLOOD COLLECTION AND STORAGE SUPPLIES

The following are specific laboratory supplies needed for collection or storage of repository specimens, which would likely not be routinely available in most laboratories.

1) 2.0 mL draw volume purple-top EDTA anticoagulated evacuated blood collection tubes: Becton Dickinson Vacutainer No. 369651 or equivalent.
2) Sterile Cryovials for storing frozen specimens - Sarstedt catalog no. 72.694/006 skirted V-bottom sterile polypropylene freezer vial with screw cap and O-ring, or equivalent.

SUPPLY ORDERING CONTACT INFORMATION

1) Becton Dickinson websites: http://www.bd.com/americas/ or http://www.bd.com/international

2) Sarstedt, Inc., P.O. Box 468, Newton, NC 28658-0468, telephone: 828-465-4000, fax: 828-465-0718. Sarstedt International website: http://www.sarstedt.com

COURIER SERVICE COMPANY – World Courier

SPECIMEN COLLECTION:

1) Infants: Collect blood by peripheral venipuncture into a 2.0 mL draw volume purple-top EDTA anticoagulated evacuated blood collection tube. Gently invert tube 8-10 times to distribute anticoagulant and to prevent clot formation.
2) Women: Collect blood by peripheral venipuncture into a 6.0 mL draw volume yellow-top ACD-B anticoagulated evacuated blood collection tube. Gently invert tube 8-10 times to distribute anticoagulant and to prevent clot formation.
3) Label all specimens with the patient identification (PID) number and the date and time of collection.

4) Transport specimen at room temperature to the laboratory for processing.

SPECIMEN PROCESSING AND STORAGE:

1. EQUIPMENT/SUPPLIES/REAGENTS

1.1 Gloves (latex, vinyl, nitrile)
1.2 Lab coat or protective gown
1.3 Anticoagulated blood
1.4 Ficoll density gradient solution (density = 1.077), sterile and endotoxin tested. Label container with date after opening. The shelf life for Ficoll is 6 months after opening. However, discard if manufacturer's expiration date occurs before this 6-month period. It is best to purchase small volumes of this reagent and replace frequently. Examples: Ficoll-Paque, Amersham-Pharmacia, cat# 17-1440-02; Sigma Histopaque-1077 Hybri-Max, cat# H8889.
1.5 Sterile Phosphate Buffered Saline (PBS), Ca++-free and Mg++-free or Sterile Hanks Balanced Salt Solution (HBSS). Observe manufacturer's outdate. Label bottle with open date; use opened bottle within three months.
1.6 Fetal Bovine Serum (FBS), heat-inactivated at 56 deg C for 30 minutes (mix larger volumes several times while inactivating).
1.7 Sterile Complete RPMI 1640 medium. Supplement RPMI 1640 medium with 2mM L-glutamine to final concentrations 100 units Penicillin/mL, 100ug Streptomycin/mL, and 10% fetal bovine serum. Medium may be filter-sterilized after addition of supplements.
1.8 Dimethyl Sulfoxide (DMS). Store at room temperature. DMSO must be fresh and sterility maintained. The shelf life for DMSO is 6 months after opening. Label with the date upon opening. Example: Hybrimax, Sigma, cat# D2650
1.9 Sterile conical centrifuge tubes, 15-mL (pediatric) or 50-mL (adult)
1.10 Sterile pipettes, graduated and transfer
1.11 Pipetting device
1.12 Sterile pipette tips
1.13 Micropipettors of various volumes
1.14 Sterile, Cryopreservation Vials (cryovials). 1.5-mL to 2-mL with screw cap, external threads, and O-rings. NOTE: Some cryovials are unacceptable for use in liquid nitrogen. Please check the manufacturer's recommendations before using. Examples: Sarstedt, cat# 72.694.005; Corning, cat# 430489
1.15 Cryo Labels specific for use in freezing and liquid nitrogen. Examples: Cryotags/Cryobabies 1.50" x 0.75", Cat # LCRY-1200; Shamrock 5/8" x 1" satin cloth labels, cat# ACTG-SCPF; Pioneer 1.75" x 0.5", cat# 710; CILS 9100 labels
1.16 Laminar flow hood (minimum class 2, Type A Biosafety hood)
1.17 Centrifuge with horizontal rotor, with speeds up to 1800 X g, and equipped with aerosol safe canisters
1.18 Automated cell counter
1.19 Insulated freezer container for cryovials: Nalgene "Mr. Frosty" (Nalgene Cryo 1 deg C Freezing container, Nalgene cat# 5100-0001; Curtis Matheson Scientific, cat# 288-383; or Fisher Scientific, cat# 15-350-50); or Cryomed Freezing Chamber (Gordinier Electronics)
1.20 -70 deg C freezer
1.21 Liquid nitrogen storage tank with LN2-rated boxes (with holes to allow LN2 drainage). Note: storage of single vials in canes is not recommended due to safety concerns (submersion in liquid phase) and possible damage to the affixed labels.

Separation and processing of plasma and PBMCs should ideally take place within four to six hours of collection, but no longer than 30 hours after collection.

To separate plasma from PBMCs:

1) Mix the blood collection tube(s) well by inverting several times.
2) Pour the whole blood into a 50 or 15 mL sterile conical centrifuge tube.
3) Centrifuge at 400 x G for 10 minutes at 24° C.
4) Remove the plasma (which should be aliquoted into labeled tubes and stored frozen per instructions below).

Plasma Processing:

1) Centrifuge the separated plasma again at 1200 x G for 10 minutes to remove any contaminating cells and platelets.
2) Plasma should then be aliquoted in sterile cryovials.
3) Aliquot twice-centrifuged plasma into 1.5 ml freezer vials.
4) Each vial will be used to store aliquots of 0.5 mL.
5) Approximately two to four 0.5 mL plasma aliquots per specimen are anticipated.
6) Label freezer vials with the PID number, date and time of collection, specimen type, and anticoagulant codes.
7) Freeze at -70° C and ship regularly (schedule to be determined) on dry ice to the central repository (see below).

PBMC Processing (after above steps to separate plasma):

1) Dilute the cells 1:2 by adding an equal volume of sterile Dulbecco's Phosphate Buffered Saline (PBS).
2) Cap tubes and invert to mix several times.
3) Under or over layer the diluted blood with a 2x volume of lymphocyte separation media (Ficoll-Hypaque).
4) Centrifuge at 400 x G for 30 minutes at 24° C in the tabletop centrifuge.
5) Aspirate off the top layer of Dulbecco's PBS until approximately 0.5 cm above the PBMC layer.
6) Remove the PBMC layer with a sterile transfer pipette and place it into a 15 mL conical centrifuge tube.
7) Add Dulbecco's PBS in a 3:1 ratio to the cells.
8) Mix well by inversion.
9) Centrifuge at 400 x G for 30 minutes at 24° C in the tabletop centrifuge.
10) Aspirate off the supernatant fraction and discard.
11) Add 10 mL of Dulbecco's PBS to the cells.
12) Resuspend the cell pellet by vigorously tapping the side of each tube with hand or by using a pipette.
13) Centrifuge the mixture for 10 minutes at 400 x G at 24° C in the tabletop centrifuge.
14) Aspirate off the supernatant fraction and discard.
15) Add 1 mL coculture medium to the cell pellet.
16) Resuspend the cell pellet by vigorously tapping the side of each tube with hand or by using a pipette.
17) Remove 0.1 mL of the cell suspension into a 75 x 100 mm tube for a white blood cell count with

an automated cell counter.

Freezing of PBMCs:

1) PBMCs at a known concentration are centrifuged at 400 x G for 10 minutes at 20 to 24° C and the supernatant is removed.
2) The PBMCs are resuspended to a concentration of 2.5×10^{6} to 1×10^{7} PBMC/mL (keep on ice) with cold Cryoprotective Medium (DSMO): RPMI 1640 containing glutamine, 10% sterile dimethylsulfoxide (DMSO) and 50% heat-inactivated fetal bovine serum (FBS), prepared fresh for each freezing procedure and cooled to 2-8° C before use. The Cryoprotective Medium is added dropwise, with constant mixing, over 1 to 2 minutes.
3) Dispense 1 mL aliquots of the cell suspension into cryovials.
4) Label freezer vials with the PID number, date and time of collection, specimen type, and anticoagulant codes.
5) Place the cryovials in a small, insulated (styrofoam) container in the bottom of a -70° C freezer for 2 to 24 hours, then transfer to vapor-phase liquid nitrogen for storage.

SPECIMEN TRANSPORTATION:

1) Transport frozen PBMC and plasma specimens to the repository on dry ice. Shipments should be sent early in the week to allow for weekday delivery at the repository.
2) Shipment of all specimens must comply with applicable U.S. Government regulations (421 CFR 72 and 49 CFR 171-8) and requirements of the International Air Transport Association (IATA)/International Civil Aviation Organization (ICAO) regarding transport of dangerous goods, including use of approved infectious substance shipping containers.
3) Contact courier service company one working day in advance of the shipping day. Courier service company staff will provide IATA-approved containers and sufficient dry ice to assure that specimen integrity is retained.
4) Send email or fax notification to the repository on the day of shipment. Notification should include the PID, specimen type, date of shipment, and anticipated date of arrival.
5) For additional instructions or questions about shipping, contact the local World Courier representative.

APPENDIX VI

NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT (NICHD)
SAMPLE INFORMED CONSENT

Full Title:
A PROSPECTIVE, OBSERVATIONAL STUDY OF HIV-INFECTED PREGNANT WOMEN AND THEIR INFANTS AT CLINICAL SITES IN LATIN AMERICAN AND CARIBBEAN COUNTRIES

Abbreviated Title:
OBSERVATION OF HIV-INFECTED PREGNANT WOMEN AND THEIR INFANTS IN LATIN AMERICAN AND CARIBBEAN COUNTRIES

Note: Clinical centers may choose to use either the full title or abbreviated title of the protocol on the site-approved informed consent document.

INTRODUCTION

You are being asked to be in this research study because you are infected with Human Immunodeficiency Virus Type 1 (HIV), and your baby is therefore exposed to HIV. This study is sponsored by the National Institute of Child Health and Human Development (NICHD), which is part of the National Institutes of Health (NIH) in the United States, and is being conducted at several sites in the Caribbean and Latin America. The doctor in charge of this study at this site is: **(insert name of Principal Investigator).** Before you decide if you want you and your baby to be in this study, we want you to know about the study.

This is a consent form. It gives you information about this study. The study staff will talk with you about this information. You may ask questions about this study at any time. If you agree to be in this study and also allow your baby to be in this study, you will be asked to sign this consent form. You will get a copy of this consent form to keep.

WHY IS THIS STUDY BEING DONE?

Acquired Immune Deficiency Syndrome , AIDS, is a disease that destroys the body's immune system (what the body uses to fight infection), leaving a person unable to fight life-threatening illnesses. The virus that causes AIDS is called the Human Immunodeficiency Virus, or HIV. The purpose of this study is to collect information about HIV-infected pregnant women and their HIV-exposed infants who are being seen at participating hospital clinical center sites. This study is called an observational study because your health information is being looked at, or observed, by your doctor and other study staff. This information will be used to better understand the safety of medicines or other treatments that are used to prevent and treat HIV in mothers and babies. You and your baby will be receiving from your doctor medicine(s) that have been approved for use in people with HIV infection. None of these medicines is being tested for the first time in this study, but are the same medicines your doctor would be prescribing for you and your baby if you chose not to be in this study. Since this is an observational study, we will be observing how you and your baby handle the medicines, and what effects the medicines may have over time.

WHAT DO I (DOES MY BABY) HAVE TO DO AT THE STUDY VISITS?

If you choose to be in this observational study, your and your baby's health information will be collected at each study visit. You and your baby will be given a patient identification number (PID #). Any information collected will be put on study forms without your or your baby's name, medical record number, or other information that could tell people outside of the study who you are or who your baby is. Some of the information to be collected from your medical chart includes: your medical and pregnancy history, including questions about your care while you are pregnant; physical exam; laboratory tests related to your HIV infection and your general health; anti-HIV medications used during your pregnancy; and, your pregnancy outcome. Information to be collected from your baby's medical chart includes: laboratory tests related to your baby's general health and to show whether or not your baby is infected with HIV; medical history and physical exam; growth measurements; medications received; and, whether or not you breastfeed your baby.

WHAT EXAMS AND LABORATORY TESTS WILL BE PERFORMED AT THE STUDY VISITS?

You will be examined by your doctor for this study, if possible, three times before your baby is born: at 8-16 weeks, 20-28 weeks, and 32-36 weeks of pregnancy. You will have a vaginal swab done at one of your visits before your baby is born. You and your baby will be examined by your doctor at delivery/birth, prior to hospital discharge, at 6-12 weeks after delivery/birth and at six months after delivery/birth. Blood will be drawn from you and your baby for any laboratory tests needed at each visit using sterile needles and technique in order to follow your and your baby's health, including testing for HIV infection in your baby. You will have about 14 ml (3 tsp) of blood drawn at each visit, and your baby will have about 8 ml (2 tsp) drawn at each visit. These blood tests will help your doctor know how well your or your baby's kidneys and liver are working, the numbers of different types of blood cells, and how well your or your baby's body fights infection. Blood will also be collected and stored for an unknown amount of time in a freezer for possible future research studies. At no time will this extra blood be drawn if it is felt that it will harm your or your baby's health in any way. These samples will be stored with your or your baby's patient identification number (PID) on them.

WHAT WILL BE DONE WITH MY (MY BABY'S) STORED BLOOD SAMPLES?

You and your baby's samples will only be used to learn more about HIV infection and its complications. The research studies that might be done on stored blood specimens could include studies to understand how HIV causes disease, and how to best treat or prevent HIV infection and its complications. Testing might include: new ways of measuring the amount of HIV in the blood; how the virus changes itself so anti-HIV medications stop working (viral resistance); tests to measure the amount of anti-HIV medications in the blood; or, new ways to know when HIV may cause symptoms of illness.

Your and your baby's stored blood samples will be looked at to help doctors understand more information about HIV and HIV treatments in HIV-infected pregnant women and their HIV-exposed infants. The future research studies to be done on your and your baby's stored blood samples collected from this study will be experimental (testing new ideas or tests), and these samples may not be looked at for many months or years after they have been collected. Results from these future studies will be provided to the doctor in charge of the study at your site who may then give them to you. You may choose to withdraw your or your baby's blood samples from the storage freezer at any time. If you do not wish to allow the study team to store these samples, you and your baby may still be in this study.

HOW MANY WOMEN/BABIES WILL TAKE PART IN THIS STUDY?

This study was not designed for a specific number of mothers and babies. It is expected that about 180-240 mother/baby pairs may take part in this study during the first year at all study sites.

HOW LONG WILL I (MY BABY) BE IN THIS STUDY?

You and/or your baby will be in this study beginning with your first visit to your doctor at this site for your pregnancy care until your baby is six months of age.

WHY WOULD THE DOCTOR TAKE ME/MY BABY OFF THIS STUDY EARLY?

The study doctor may need to take you (your baby) off the study early without your permission if:

- You (your baby) are not able to come to the study visits as required by the study; or
- The NICHD or your country's national/local regulatory authorities, such as your country's Ethics Committee, stop the study.

WHAT ARE THE RISKS OF THIS STUDY?

There are no major risks to being in this study. The possible risks of blood drawing include discomfort, bleeding, and/or bruising where the needle enters the body, and, in rare cases, fainting or infection.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

There are no direct benefits to you or your child from taking part in this study. Information learned from this study may help doctors better understand the risks of HIV medications taken to improve the mother's health or to prevent HIV from being given to babies from their mothers. Also, this study may help doctors better understand the risks related to using cesarean section to prevent mothers from giving HIV to their babies.

WHAT OTHER CHOICES DO I (DOES MY BABY) HAVE BESIDES THIS STUDY?

This study does not provide any medication. If you choose, for yourself and/or for your baby, to not be in this study, you and your baby will continue to receive whatever treatment is provided at your clinical site and will be checked by your doctor at the schedule he or she recommends.

WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep your and your baby's medical records confidential (private), but complete confidentiality cannot be guaranteed. Your and your baby's medical records may be opened if required by law. Results of tests performed with your and your baby's blood samples will be kept confidential. In addition, all records will be kept in a locked file cabinet. However, individuals carrying out this study may see these records and results of this study may be published in scientific journals. You and your baby will not be personally identified in any publication that results from the information collected in this study, since you and your baby will only be identified by a patient identification number (PID). Individuals who might have access to your and your baby's records include doctors from NICHD, national/local country regulatory authorities, study staff, or study monitors (people who make sure the study is done the correct way) who work with NICHD.

WHAT ARE THE COSTS TO ME?

There is no cost to you for the study-related clinic visits, examinations, or laboratory tests. Any medical costs for you and your baby's treatment outside of this study will be charged to you or your health insurance.

WILL I RECEIVE ANY PAYMENT?

Also, you and your baby will not receive any payment for being in this study.

Note: It is acceptable for clinical centers to assist families with transportation and meals by providing incentive payments. Such payments may be mentioned in this section of the informed consent document.

WHAT HAPPENS IF I AM (MY BABY IS) INJURED?

If you or your baby is injured as a result of being in this observational study, the **(insert name of the clinic)** will give you or your baby immediate necessary treatment for the injury. The cost for this treatment will be charged to you or your insurance company. You will then be told where you or your baby may receive additional treatment for injuries. There is no program for payment to you either through this institution or the National Institutes of Health (NIH). However, you will not be giving up any of your legal rights by signing this consent form.

WHAT ARE MY (MY BABY'S) RIGHTS AS A RESEARCH SUBJECT?

Taking part in this study is completely voluntary. You may choose not to, or not to allow your baby to, take part in this study at any time. You may take yourself and your baby out of the study at any time. You and your baby will be treated the same no matter what you decide.

WHAT ABOUT NEW FINDINGS OR STUDY RESULTS?

Any important findings learned during the study will be given to you by a study doctor (or study staff member) at your site. At the end of the study, you will be told when study results are ready and how to learn about them.

WHAT DO I DO IF I HAVE PROBLEMS OR QUESTIONS?

For questions about this study or a research-related injury, contact:

- **[name of the investigator or other study staff;**
- **telephone number of above.]**

For questions about your and your baby's rights as a research subject, contact:

- **[name or title of person on your country's Ethics Committee or other similar organization for this site;**
- **telephone number of above.]**

STATEMENT OF CONSENT

I have read this consent form (or someone has explained it to me), all of my questions have been answered, and I agree to take part in this study. I understand that I may take myself and/or my baby out of this study at any time and I and/or my baby will still have rights to receive medical care.

_____	_____	_____
Participant's Name* (print or type)	Participant's Signature*	Date

*Participant's name or name of legal representative or guardian, as appropriate.

I agree to have my and my baby's blood samples stored for future research studies.

Yes No (Circle one.)

_____	_____	_____
Study Staff Conducting Consent Discussion (print or type)	Study Staff Signature	Date

_____	_____	_____
Witness' Name (print or type) (as appropriate)	Witness' Signature	Date

APPENDIX VII
EXACT 95% CONFIDENCE INTERVAL CALCULATION

The exact 95% confidence interval for a given sample size and prevalence, P_v, is based on the relationship between the binomial and $F-$distribution[1]. More specifically, suppose a certain characteristic appears in m subjects in a sample of size n then the prevalence is estimated as $\hat{P}_v = \frac{m}{n}$ with exact 95% confidence limits given by

$$\left[\frac{m}{m+(n-m+1)F_{0.025,d_1,d_2}}, \frac{(m+1)F_{0.025,d_2+2,d_1-2}}{n-m+(m+1)F_{0.025,d_2+2,d_1-2}} \right]$$

where $d_1 = 2(n-m+1), d_2 = 2m$

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[1] Fisher RA, Yates F. Statistical tables for biological, agricultural, and medical research. 6th edition. New York: Hafner, 1963: 146.