NISDI Pediatric Latin American Countries Epidemiologic Study (PLACES)

A PROSPECTIVE, OBSERVATIONAL STUDY
OF HIV-INFECTED CHILDREN
AT CLINICAL SITES IN LATIN AMERICAN COUNTRIES

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PRECIS

This is an observational, prospective cohort study to describe the demographic, clinical, immunologic, and virologic characteristics of HIV-infected children at participating clinical sites in Latin America countries. Enrollment in this study will consist of approximately 500 HIV-infected children in two cohorts who acquired HIV infection through mother-to-child transmission (MTCT). The first group will be a static cohort consisting of HIV-infected children who were five years of age or younger when previously enrolled into the NISDI Pediatric Protocol. The second cohort will be a dynamic cohort of prospectively enrolled, HIV-infected children who are five years of age or younger. We will characterize complications from both the disease and its treatments. Subjects will be evaluated every six months for approximately five years and assessments of growth, morbidity, disease progression and mortality will be made.

SCHEMA

A PROSPECTIVE, OBSERVATIONAL STUDY OF HIV-INFECTED CHILDREN AT CLINICAL SITES IN LATIN AMERICAN COUNTRIES

DESIGN: Prospective cohort study to describe the characteristics of HIV-infected children followed at participating Latin American sites and to evaluate early and late outcomes in these children.

SAMPLE SIZE AND POPULATION:

Static Cohort: Children who were enrolled into the NISDI Pediatric Protocol prior to their 6th birthday, who acquired HIV infection through mother-to-child transmission (MTCT), and who receive medical care at participating clinical sites.

Dynamic Cohort: HIV-infected children enrolled prior to their 6th birthday, who acquired HIV through MTCT, and who receive medical care at participating clinical sites.

REGIMEN: Prospective data collection, to include history, physical examination, laboratory evaluations (including hematology, flow cytometry, biochemistries, and HIV-specific viral assays), and assessment of growth, morbidity, and mortality. At baseline, retrospective assessment of antiretroviral (ARV) exposure, historical HIV-related diagnoses, CD4 counts and viral loads will be recorded.

STUDY DURATION: HIV-infected children will be followed for a minimum of one year, and potentially up to five years.

PRIMARY OBJECTIVES:

1) To characterize ARV treatment and/or other therapies for HIV infection or its complications in HIV-infected children at participating clinical sites in Latin America.

2) To describe the demographic, clinical, immunologic, and virologic characteristics of HIV-infected children followed at participating clinical sites in Latin America.*

3) To characterize early and late outcomes related to HIV disease among HIV-infected children followed at participating clinical sites in Latin America.*

4) To characterize early and late outcomes of *in utero* and postnatal exposure to ARV treatment among HIV-infected children*.

* Parameters to be evaluated include types of therapy; growth; morbidity, including organ system toxicity; incidence and prevalence of opportunistic infections and malignancies; and immunologic and virologic parameters and mortality of the study population.

1.0 INTRODUCTION

UNAIDS estimates that there are 1.6 million adults and children living with human immunodeficiency virus type 1 (**HIV)** infection in Latin America countries, of whom 512,000 are women of childbearing age and children (numbers that are generally regarded as underestimates). In 2006, fewer than 43,000 adults and children were newly infected with HIV in North America, as compared with almost 140,000 adults and children in Latin American countries (1). Finally, while 68 percent of all people requiring **ARV** therapy in Latin America and the Caribbean are receiving it, it is estimated that only 8 percent of children who require **ARV** therapy are receiving it (2)**. HIV/AIDS** is now the leading cause of death (followed by cardiovascular diseases) in countries in Latin America and the Caribbean.

The epidemiology of mother-to-child transmission (MTCT) of HIV in resource rich-settings has markedly changed over the years with implementation of interventions to prevent MTCT of HIV. Successful interventions have included ARV prophylaxis, cesarean delivery before labor and before rupture of membranes, and complete avoidance of breastfeeding. Use of highly active combination ARV regimens to treat HIV disease has significantly decreased morbidity and prolonged the life of infected individuals, and when used by HIV-infected pregnant women, may further decrease the risk of MTCT. Despite these successes, children in Latin American countries continue to become HIV infected and many questions remain concerning the safety of exposure and long term treatment with combination ARV regimens in HIV-infected infants and children. The short-term safety of these interventions and treatments has been demonstrated in clinical trials. However, the late effects of chronic treatment are still being defined. This study will address changes in the natural history of ARV-treated HIV disease as survival increases, as well as the long term consequences of ongoing exposure to multiple ARV agents and drugs used for the prophylaxis and treatment of opportunistic infections in HIV-infected children.

Several descriptive studies designed to assess changes in the natural history of HIV infection and the effects of potent combination ARV treatment in children have been conducted in the United States. These studies include: PACTG 219, a late outcomes protocol following infected and uninfected children through age 24 years; the Women and Infants Transmission Study (WITS), a natural history study of HIV-infected women and their exposed infants; and the Pediatric Spectrum of Disease (PSD) project, a surveillance study performed by the Centers for Disease Control and Prevention. Both PACTG 219C and WITS are now closed and analysis is continuing. New or ongoing initiatives that attempt to address various aspects of these issues in HIV-infected children include the Pediatric HIV/AIDS Cohort Study (PHACS) to be conducted in the United States; Legacy (a CDC surveillance study which is also U.S.-based); and the European Collaborative Study of pregnant HIV-infected women and their children. In addition, the newly formed International Epidemiological Databases to Evaluate AIDS (IeDEA), whose pediatric component incorporates the KIDS-ART-LINC collaboration, an African pediatric cohort, and the TREAT ASIA Pediatric HIV Network, will allow the collection of clinical and laboratory data on HIV-infected children in sub-Saharan Africa, Asia, and Australia. The NISDI Pediatric Latin American Countries Epidemiologic Study (PLACES) Protocol may contribute to these cohorts by providing information about Latin American children with HIV infection.

Many countries in Latin America have initiated programs of ARV treatment for HIV-infected children. Over the last 5 years, NICHD has expanded its international collaborations in countries in Latin America and the Caribbean through funding of two prospective cohort studies, the NICHD International Site Development Initiative (NISDI) project, which began enrollment in 2002. The NISDI Perinatal Study has enrolled 1220 HIV-infected pregnant women and followed these women and their HIV-exposed infants until 6 months after delivery/birth. The NISDI Pediatric Study has enrolled 1605 HIV-exposed and HIV-infected infants, children, and adolescents. This amended

protocol (PLACES), to begin enrollment in late 2007 will focus on a subset of the population identified in the NISDI Pediatric Study. It will be a prospective cohort study to assess HIV-infected children previously enrolled into the Pediatric NISDI Protocol before age 6 and newly identified children before their 6th birthday who have become HIV infected through MTCT, for up to 5 years of follow up.

The conduct of this prospective, multicenter, observational study of HIV-infected children (PLACES) at sites in Latin America will provide important data about the demographic, clinical, immunologic and virologic characteristics of this population. In addition, this study will be critical in providing information about the safety and long term effects of ARV or other therapeutic drug exposures among HIV-infected children in these countries.

1.1 Background

1.11 Prevention of Mother-to-Child Transmission of HIV

Over 90 percent of pediatric HIV infection is acquired through MTCT (during pregnancy, at birth or postnatally through breastfeeding). Data obtained prior to the institution of interventions to prevent MTCT of HIV indicated overall MTCT rates ranging from 12-42 percent, with the highest rates of transmission observed among breastfeeding populations (3).

Over the past several years, major successes have been achieved in prevention of MTCT of HIV. For example, the estimated number of perinatal HIV infections in the U.S. has decreased from a peak of 1650 infections in 1991 (4) to an estimated 144-236 infections in 2002 (5). Similarly, the estimated number of perinatally acquired cases of AIDS in the U.S. peaked in 1992 (945 cases), but subsequently decreased by 95 percent by 2004 (48 cases) (6). The decreases in the number of perinatally acquired HIV infections and AIDS cases most likely represent improved identification of HIV-infected mothers and their exposed infants, and the utilization of appropriate interventions to prevent transmission. Observed rates of MTCT have decreased to less than 2 percent with the use of efficacious interventions to prevent transmission, compared with transmission rates of 25-30 percent or more without any intervention (7,8). However, such successes have occurred primarily in those countries with the greatest resources and the lowest burden of HIV infection among women and children. Significant challenges remain, particularly in those countries with more limited resources and a greater population burden of HIV infection (1).

1.12 Antiretroviral Treatment of HIV-Infected Children

The advent of highly active antiretroviral therapy (HAART) has dramatically altered the course of HIV infection in HIV-infected adults and children in the United States and other countries where ARV therapy has been incorporated into clinical practice. Since the late 1990s, HAART has been increasingly used for treatment of infected children in more developed countries. As observed in infected adult populations treated with HAART, significant decreases in hospitalizations, use of antibiotics, gains in life expectancy, and decreases in opportunistic infections have been observed in children treated with HAART (9, 10). Data on protease inhibitor use and mortality in HIV-infected children between 1995 and 1998 was evaluated in PACTG 219, a late outcomes study. Prior to 1996, no protease

inhibitor use was reported in enrolled children; by 1998, this had increased to over 70 percent. This increase in use of protease inhibitors was accompanied by a substantial reduction in mortality in all age groups, from 5 percent in 1996 to 2.1 percent in 1997, 0.9 percent in 1998, and 0.7 percent in 1999 (9). Similar data have been reported from the U.S.-based Pediatric Spectrum of Disease study (10) and from Italy (11). Additionally, some complications of HIV infection in children, such as growth failure, have seen significant improvements with HAART (12).

In some clinical **sites** in Latin America, **ARV** treatment, including HAART, is provided for HIV-infected individuals, including children. In Central America, access to ARV therapy is more limited than it is in South America.

Changes in the natural history of pediatric HIV disease with increasing use of ARV therapy similar to those in the United States have been observed in Latin America. A study from Sao Paulo, Brazil of 1,066 perinatally infected children evaluated the relative hazard of death by age 18 months for children diagnosed in the time periods of 1988-1991 and 1992-1994 compared to those diagnosed in 1987 and earlier. The hazard of death for children diagnosed during 1988-1991 was 0.59 (95% confidence interval [CI] 0.37-0.96) when compared to children diagnosed in 1987 and for those diagnosed between 1992-1994, when ARV therapy become available, the relative hazard of death was 0.45 (95% CI 0.28-0.72) when compared to children diagnosed in 1987 (13). More recent data from Sao Paulo shows a decrease in reported AIDS cases of 59% from 1987 and a decrease in deaths from AIDS from 164 in 1994 to 20 in 2002 in perinatally infected children (14). In Buenos Aires, Argentina, significant decreases in mortality and progression to AIDS in HIV-infected children have been observed since 1996, when combination ARV therapy was initiated. In 1990-1995, overall mortality in infected children was 30 percent compared to 18 percent in 1996-1999; 5-year survival was 71 percent for 1990-1995 compared to 84 percent for 1996-1999 (15).

The types of opportunistic infections observed in HIV-infected children will reflect geographic differences in the prevalence of endemic pathogens. Hence, HIV disease manifestations and changes in the natural history of disease in children with improvements in treatment and prophylaxis will be reflected differently in different countries. Endemic diseases such as tuberculosis and causes of persistent diarrhea such as cryptosporidia occur more frequently in Latin America than in the United States, and can cause opportunistic infections in HIV-infected children in these countries (16, 17). Administration of BCG vaccine at birth is standard practice in some Latin American countries, and disseminated infection with *M. bovis*-BCG has been reported in 2 percent of 645 HIV-infected children in Argentina (18). Thus, changes in the natural history of HIV disease with treatment in Latin America will differ from those in other countries, and need to be evaluated separately.

1.13 Issues Related to Late Effects of HIV Disease and Antiretroviral Exposure of Infected Children

The effect of *in utero* ARV drug exposure on infants who become infected despite ARV prophylaxis is controversial. In several cohort studies, infants who became infected despite ZDV prophylaxis were observed to have more rapid disease progression than infected infants born to mothers not receiving prophylaxis, although response to potent therapy was similar between infected infants with and without *in utero* exposure in one of these cohorts (20, 21, 22). In contrast, in the randomized, controlled clinical trial PACTG 076, HIV disease progression and viral replication did not differ between infected ZDV-exposed and placebo infants, and ZDV resistance was not detected in ZDV-exposed infected infants, despite receipt of 6 weeks of ZDV prophylaxis (23). As most children enrolled into this protocol will have

been exposed to different combinations of *in utero* ARVs, the effects of exposure to different types of regimens will be explored and described.

In adults treated with long term HAART, a number of late adverse effects of treatment have been observed, including hyperlipidemia, lipodystrophy, insulin resistance, hyperglycemia, osteopenia, and disorders related to mitochondrial dysfunction (24). Cardiomyopathy, peripheral neuropathy, pancreatitis, anemia, hepatic steatosis and lactic acidosis have been described. Some manifestations resolve after treatment discontinuation and a genetic susceptibility may be involved (19). Reports of similar disorders in infected children receiving HAART have been published (25, 26, 27, 28, 29, 30).

Both HIV infection and ART have been shown to adversely influence glucose and lipid metabolism (31, 32, 33). Current data suggest that there are associations between all three widely used classes of ART and potentially deleterious alterations in lipid metabolism, including hypertriglyceridemia and increased levels of total and LDL cholesterol (33, 34, 35, 36, 37, 38, 39, 40). Likewise, abnormalities of glucose metabolism, including impaired glucose tolerance, hyperinsulinemia, and insulin resistance, have been associated with treatment with some PIs (33, 37, 40) and some NRTIs (41, 42).

A study from Rio de Janeiro retrospectively compared lipid changes in 66 HIV-infected children receiving ARV therapy with and without protease inhibitors (43). Lipid metabolic changes occurred in over 50 percent of children, with a similar frequency in children receiving or not receiving protease inhibitors; longer HIV disease duration correlated with the risk of serum lipid changes. In contrast, lipodystrophy occurred significantly more frequently in children receiving protease inhibitor-containing therapy. The NISDI Pediatric Protocol found an overall prevalence of 15 percent of hypercholesterolemia and 26 percent of hypertriglyceridemia in HIV-infected children from Mexico, Argentina and Brazil, results similar to these seen in a large U.S. cohort. There was an increased risk of both hypertriglyceridemia and hypercholesterolemia with use of PIs (44).

While antiretroviral drug treatment has changed HIV into a chronic disease, short and long term adverse effects of treatment have been observed. In infants and children who are still growing, the long term effects of ARV drugs and other treatments may have an even greater effect than in adults who have completed growth and development.

While descriptive studies are ongoing in the United States to assess the long term effects of HIV treatment on infected children, data regarding late outcomes in Latin American countries are generally not available. The institution of long term follow-up studies in Latin America will expand the available data on late effects and broaden the scope of knowledge regarding benefits and potential toxicities of ARV therapies for infected children.

1.14 Pediatric and Perinatal HIV Clinical Trials

The **Pediatric AIDS Clinical Trials Group (PACTG), now the International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT),** conducts the vast majority of pediatric and perinatal HIV clinical trials in the U.S. IMPAACT 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, data processing, as well as development and management of specific protocols. The NIAID funds IMPAACT 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.

In 2002, the NICHD expanded its international collaborations in Latin America and the Caribbean through subcontracts with Westat for the conduct of **the two observational NISDI protocols which were** multicenter, prospective cohort stud**ies** of HIV-infected **women and their exposed infants and** children. **Fifteen clinical sites in Brazil, Argentina, and Mexico were funded to perform the NISDI Pediatric Protocol, a prospective, observational study of HIV-exposed infants less then 12-months old, and of HIV-infected infants, children, and adolescents. In 2005, sites in Jamaica and Peru were funded to participate. Funding for these two NISDI protocols will conclude in October 2007.** NICHD is amending the current NISDI Pediatric Protocol to include only a subset of children. Specifically, PLACES will enroll only HIV-infected children, infected through MTCT and from Latin American countries. This prospective, observational study will provide additional information on the short and long term effects of disease and antiretroviral treatment to Latin American countries in which antiretroviral treatment is available Findings from this study will **inform research of HIV-infected children enrolled at less then 6 years of age and further** the development of future clinical trials.

Intercurrent conditions identified during the course of these protocols that might affect the health or well-being of children in this study will receive appropriate follow-up and care at the clinical **sites** in which the study is being conducted, as the **sites** are providing full clinical care, not solely HIV-related care, to these patients. Referral to appropriate specialists may occur if the condition is outside the expertise of the investigators.

1.2 Study Rationale

The short-term safety and efficacy of **ARVs for prevention of MTCT and for the treatment of HIV infection in children has been demonstrated in clinical trials. However, the late effects of these interventions, particularly after prolonged exposure, are not yet fully understood. The continued prospective assessment of young children exposed to ARVs is critical to ensure a thorough evaluation of potential late effects of these potent agents.** This study seeks to obtain descriptive data regarding the general health status of HIV-infected children **in Latin American countries** to evaluate the incidence of currently known effects of treatment**,** and to increase the capability of surveillance for as yet undescribed effects of treatment.

The study will be a prospective, multicenter, observational study to describe the characteristics and early and late outcomes HIV-infected children at participating sites in Latin America where **ARV** treatment **for HIV-infected children is** available.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.11 **To characterize ARV treatment and/or other therapies for HIV infection or its complications in HIV-infected children at participating clinical sites in Latin America.**

2.12 **To describe the demographic, clinical, immunologic, and virologic characteristics of HIV-infected children followed at participating clinical sites in Latin America.***

2.13 To characterize early and late outcomes related to HIV disease **among** HIV-infected children followed at participating clinical sites in Latin America.*

2.14 **To characterize early and late outcomes of *in utero* and postnatal exposure to ARV treatment among HIV-infected children*.**

* Parameters to be evaluated include types of therapy; growth; morbidity**, including** organ system toxicity**;** incidence **and prevalence** of opportunistic infections and malignancies; and immunologic and virologic parameters **and mortality** of the study **population.**

3.0 STUDY DESIGN

This prospective, multicenter, observational study will describe the early and late outcomes of HIV disease and its treatment in young HIV-infected children at participating sites in Latin American countries where pediatric ARV treatment is available.

The study population will be divided into two cohorts:

1. Static cohort: comprises children who were enrolled into the NISDI Pediatric Protocol prior to their 6th birthday, who acquired HIV infection through MTCT, and who receive medical care at participating clinical sites:

2. Dynamic cohort: comprises HIV-infected children prior to their 6th birthday, who acquired HIV through MTCT, and who receive medical care at participating clinical sites.

Both groups of children will be evaluated at 6-month intervals with a clinical assessment (history and physical examination), immunologic and virologic assays, and other laboratory assessments (including hematologic and biochemical). Assessments of growth, morbidity, and mortality will be prospectively made. Historical data regarding HIV-related diagnoses, CD4 counts, viral loads and *in utero* and postnatal antiretroviral exposure will be made through medical record abstraction at the time of enrollment. Subjects will be followed for up to 5 years.

4.0 SUBJECT SELECTION AND ENROLLMENT

4.1 Inclusion Criteria

4.11 **Static Cohort**:

1. Previous participation in a NISDI Protocol
2. Less then 6 years of age (before their 6th birthday) at the time of enrollment into the NISDI Pediatric Protocol
3. HIV-infected
4. HIV infection must be documented in the medical records by:
   a. For children <18 months old when tested, two or more of the following (separate determinations on separate blood specimens):
      1. Positive HIV culture
      2. Positive HIV DNA PCR
      3. Positive neutralizable p24 antigen
      4. Quantitative HIV RNA ≥ 10,000 copies/ml
   b. For children ≥ 18 months old when tested, two or more of the following (separate determinations on separate blood specimens):
      1. Reactive test for HIV antibody in a sample obtained at ≥ 15 months of age with confirmatory test by Western Blot or Immunofluorescence assay
      2. Positive HIV culture
      3. Positive HIV DNA PCR
      4. Positive neutralizable p24 antigen
      5. Quantitative HIV RNA ≥ 1,000 copies/ml
5. Documentation of maternal HIV infection by country appropriate National Guidelines
6. Signed informed consent from parent or legal guardian. An informed assent document will be provided for children 8 years of age or older when appropriate.
7. Subjects must be able to be followed at a participating clinical site.
8. Subjects may be co-enrolled in clinical trials for treatment of HIV infection, opportunistic infections, or other HIV-related effects.

4.12 **Dynamic cohort**:

1. HIV-infected less then 6 years of age (before their 6th birthday) at enrollment into this protocol
2. HIV infection documented by:
   a. For children <18 months old when tested, two or more of the following (separate determinations on separate blood specimens):
      1. Positive HIV culture
      2. Positive HIV DNA PCR
      3. Positive neutralizable p24 antigen
      4. Quantitative HIV RNA ≥ 10,000 copies/ml
   b. For children ≥ 18 months old when tested, two or more of the following (separate determinations on separate blood specimens):
      1. Reactive test for HIV antibody in a sample obtained at ≥ 15 months of age with confirmatory test by Western Blot or Immunofluorescence assay

2. Positive HIV culture
3. Positive HIV DNA PCR
4. Positive neutralizable p24 antigen
5. Quantitative HIV RNA ≥ 1,000 copies/ml

3. Documentation of maternal HIV infection by country appropriate National Guidelines.
4. Signed informed consent from parent or legal guardian. An informed assent document will be provided for children 8 years of age or older when appropriate.
5. Subjects must be able to be followed at a participating clinical site.
6. Subjects may be co-enrolled in clinical trials for treatment of HIV infection, opportunistic infections, or other HIV-related effects.

4.2 Exclusion Criteria

4.21 Children who are born to an HIV-infected mother, but are uninfected or of indeterminate HIV infection status

4.22 Children who are orphans without legal guardians or are wards of the state

4.3 Enrollment Procedures

Prior to the collection of any data, parents or legal guardians will be provided ample time to review the informed consent document, have questions answered, and discuss any concerns related to study participation. An informed assent document will be provided for children 8 years of age or older when appropriate.

Static cohort: Subjects will be invited to participate in this cohort if they meet the following criteria: previous participation in a NISDI protocol; less then 6 years of age at the time of enrollment in the NISDI Pediatric Protocol; acquired HIV infection through MTCT; and receive medical care at a participating clinical site.

Dynamic cohort: Subjects will be invited to participate in this cohort if they have acquired HIV-infection through MTCT, are less then 6 years of age, and receive medical care at a participating clinical site

5.0 CLINICAL AND LABORATORY EVALUATIONS

Following informed consent and registration into the study, children will be seen for a baseline evaluation. Following the baseline visit, visits will be scheduled bi-annually as close as possible to their birthday and six months beyond. The acceptable window for study visits is +/- 3 months (90 days). Missed visits should be rescheduled as soon as possible.

Follow-up visits will be scheduled according to the participant's age at enrollment. For example: if a participant is enrolled at 14 months of age (baseline visit), in order to synchronize future study visits with his/her age, the next follow-up visit should be scheduled at 18 months of age. See chart below for other examples.

Age at Enrollment into PLACES (BSL Visit)	*Age at follow up visits									
	6m	12m (1y)	18m (1 ½y)	24m (2y)	30m (2 ½y)	36m (3y)	42m (3 ½y)	48m (4y)	54m (4 ½y)	F/U Every 6 m
3-9m*		x	x	x	x	x	x	x	x	x
10-15m			x	x	x	x	x	x	x	x
16-21m				x	x	x	x	x	x	x
22-27m					x	x	x	x	x	x
28-33m						x	x	x	x	x
34-39m							x	x	x	x
40-45m								x	x	x
46-52m									x	x

***Note: Follow up (F/U) visits will be scheduled according to the age of the participant**

5.1 Baseline and Ongoing Evaluations

Subjects will be evaluated at baseline and then every 6 months for the duration of the protocol at the site for the following parameters.

5.11 All children enrolled, regardless of dynamic or static cohort

5.111 History:

- Major non-HIV and HIV-related medical diagnoses;
- Complete data regarding maternal medications received during pregnancy available through medical record abstraction will be obtained at baseline only;
- Maternal co-infections during pregnancy (syphilis, toxoplasmosis, etc.) will be ascertained at baseline only;
- Subject's ARV history (including reason for change, use of generic or patent drug(s), and fixed dose combinations);
- Vaccination history;
- Historical T Lymphocyte subsets (percentage and absolute value for CD4+ and CD8+ cells);
- Historical Plasma HIV-1 RNA concentrations (viral assays);
- CDC HIV Disease Classification
- WHO Disease Classification

5.112 Physical examination, to include the following:

- Height and weight;
- Head circumference (for children ≤ 3 years of age);
- Triceps skinfold thickness;
- Mid-upper arm circumference; and
- Tanner staging for boys ≥ 9 and girls ≥ 7.

5.113 Laboratory studies:

- Hematology—complete blood count and differential with platelets;
- T Lymphocyte subsets (percentage and absolute value for CD4+ and CD8+ cells);
- HIV-1 quantitative RNA assay;

- Biochemical assays: Aspartate aminotransferase (AST [SGOT]), alanine aminotrasferase (ALT [SGPT]), total bilirubin, LDH, lipase, BUN, creatinine, albumin, total protein, creatine phosphokinase (CPK), fasting cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides;
- Fasting serum glucose and insulin levels will be obtained annually in all subjects 5 years of age and older. If the calculated homeostatic model assessment insulin resistance (HOMA-IR=(fasting insulin x fasting glucose)/22.5) is greater than 2.5 in children (Tanner stage less than 2) or greater than 4.0 in adolescents (Tanner stage greater than or equal to 2), then a 2-hour oral glucose tolerance test (OGTT) and an HbA1c will be recommended to the site investigator;
- Calculated Creatinine clearance using Schwartz formula: CrCl= k*H/Cr where k=0.45 for term infants < 1 year and 0.55 for children, H=height (cm), and Cr= serum creatinine (mg/dl).
- Genotypic or phenotypic drug resistance testing results, including method and interpretation will be collected if performed for clinical care outside protocol.

5.114 Specimen storage:

- Plasma and peripheral blood mononuclear cell (PBMC) samples for storage will be obtained from participants at entry and every visit. 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 sub-studies relating to HIV infection and its complications.
- Research sub-studies that might be performed using stored blood specimens could include studies to understand how HIV causes disease, and how to best treat HIV infection and its complications. Specific sub-studies might include new ways of quantifying HIV; measures of viral resistance and factors that might affect resistance; measures of ARV drug concentrations and biological factors that might affect these concentrations; new correlates of disease progression and new measures of toxicity. Sub-studies may also include new ways of measuring effects of long term drug exposure such as mitochondrial assays, measurement of markers of metabolic syndromes and dyslipidemias. Subjects will be asked to sign a separate informed consent form for collection and storage of their specimens and may choose not to have blood stored for future studies. Results of these studies will not be provided to the subjects as they are exploratory in nature. Should an important clinical finding that affects that subject be identified, the subject's physician will be notified. If germline testing on any specimen is proposed for future studies, additional informed consent of subjects will be required.

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

5.12 Death:

Attempts to secure documentation regarding the death of children on study will be made. Relevant documentation includes the following:

- Study-specific death form (from case report form book);
- Hospitalization records for terminal event, when applicable;

- Autopsy report, when available;
- Documentation of contact with health care provider or family member regarding time and cause of death; and
- Interview with the primary caregiver of the patient.

6.0 STATISTICAL CONSIDERATIONS

6.1 General Design Issues

This observational study is designed to collect demographic, medical history, clinical, and laboratory data at baseline and at subsequent clinic visits from HIV-infected children, enrolled prospectively during the study period. Participating clinic sites are asked to enroll 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 ARV treatment available for all HIV-infected children who meet national requirements for needing treatment. This study is designed primarily to estimate the prevalence and incidence or other parameters of the study population's clinical characteristics, and is not designed for formal hypothesis testing. Nevertheless, comparisons between subgroups of subjects may be made when statistically reasonable.

The analyses will characterize the early and late outcomes related to exposure and chronic treatment with ARVs. The following information will be evaluated: medical history, including major diagnoses; physical exam findings; growth; laboratory evaluations; virologic and immunologic assessments and mortality. These outcomes will be measured at baseline and every 6 months. While most infants born to HIV-infected women will have been ARV exposed, some infants may be enrolled without such exposure due to late identification of maternal infection status. Thus, some comparisons may be made between children who have had *in utero* ARV exposure and those infants and children who were not exposed to ARVs. In children treated with ARVs, results will be presented separately by type of ARV use including different class and individual combinations of ARVs where appropriate. For these analyses, variables may be created based on the subjects' current therapy and previous exposure (e.g., ZDV taken by mother during pregnancy) and used to define comparison groups. Outcomes may be examined according to baseline demographic characteristic (e.g., gender) or clinical characteristics (e.g., birth weight). Study data may also be compared to regional or national statistics, when available. Additionally, statistical summaries of outcome measures may be presented separately for different levels of viral load and CD4 at baseline. Analyses may examine the relationship of age to the outcome measures. Other analyses may compare known or newly described toxicities, such as hyperlipidemia or renal disease by type of antiretroviral therapy the child has received for treatment of disease or its complications.

6.2 Sample Size and Accrual

Although this study is not designed to test predefined hypotheses, some projections can be made about the potential number of study participants, and the corresponding precision of estimates and power of comparisons that might be calculated from a given sample size. In terms of the static cohort, as of March 05, 2007, there have been 1,609 infants, children, and adolescents enrolled into the NISDI Pediatric Protocol from 17 international sites in Latin American and the Caribbean. Among these subjects, approximately 809 are HIV-infected and 320 are less then 6 years of age and potentially eligible for the static cohort._Only subjects

followed at currently funded clinical sites will be eligible. Up to 200 children may be enrolled in the dynamic cohort but this number may be increased depending on funding. With this amendment the accrual ceiling will now be 2500 (2000 on the original version plus 500 on this amended version).

Databases documenting the rates of specific abnormalities (such as hyperlipidemia and growth failure) in children in Latin American countries will be sought for comparison. However, for very rare events it is likely that no events will be observed in the study group. For example, if the prevalence of a condition is 1 per 1000, then there is a 74 percent chance that no events will be observed in a group of 300 children. If the prevalence is 1 per 250, there is still a 30 percent chance of no events occurring in a group of 300 children. If no events are observed among 300 children, the upper 95 percent confidence interval for the prevalence will be approximately 1 percent.

The confidence interval associated with an estimated prevalence will vary according to the value of the estimate and number of subjects that were used in the calculation. The following table displays the 95 percent confidence intervals for estimated prevalence between 1 percent and 50 percent based on the number of subjects being between 100 and 1000.

Table 1. 95 percent Confidence Intervals for Estimates of Prevalence (P) based on Specified Number of Subjects

	Number of Subjects				
P (%)	100	200	300	500	1000
1	(0.0- 5.4)	(0.1- 3.6)	(0.2- 2.9)	(0.3- 2.3)	(0.5-1.8)
2	(0.2- 7.0)	(0.5- 5.0)	(0.7- 4.3)	(1.0- 3.6)	(1.2-3.1)
3	(0.6- 8.5)	(1.1- 6.4)	(1.4- 5.6)	(1.7- 4.9)	(2.0-4.3)
4	(1.1- 9.9)	(1.7- 7.7)	(2.1- 6.9)	(2.5- 6.1)	(2.9-5.4)
5	(1.6-11.3)	(2.4- 9.0)	(2.8- 8.1)	(3.3- 7.3)	(3.7-6.5)
10	(4.9-17.6)	(6.2-15.0)	(6.8-14.0)	(7.5-13.0)	(8.2-12.0)
15	(8.6-23.5)	(10.4-20.7)	(11.2-19.6)	(12.0-18.4)	(12.8-17.4)
20	(12.7-29.2)	(14.7-26.2)	(15.6-25.0)	(16.6-23.8)	(17.6-22.6)
30	(21.2-40.0)	(23.7-36.9)	(24.9-35.5)	(26.0-34.2)	(27.1-32.9)
40	(30.3-50.3)	(33.2-47.1)	(34.4-45.8)	(35.7-44.4)	(36.9-43.1)
50	(39.8-60.2)	(42.9-57.1)	(44.2-55.8)	(45.5-54.5)	(46.9-53.1)

Shaded entries correspond to confidence interval widths narrower than 10 percent.

For some outcomes, such as opportunistic infections, the incidence rate will be a more relevant measure because an individual patient may have more than one such infection over the course of

the study, and patients will be followed for varying amounts of time. The number of person-years will be the denominator for incidence rate calculations.

As a conservative estimate, subjects are likely to average at least 2 years of follow-up. Therefore, there should be at least **1,000** person-years of follow-up for the total study group

In Table 2, 95 percent confidence intervals for observed incidence rates of 1, 5, 10 and 20 per 100 person-years based on different numbers of person-years of follow-up are given.

Table 2. 95 percent Confidence Intervals by Incidence Rate and Follow-up Time

	Number of Person-Years of Follow-up				
Incidence Rate (per 100 person-years)	50	100	500	1000	2500
1	(0.0 - 5.7)	(0.0 - 3.9)	(0.3 - 2.1)	(0.5 - 1.7)	(0.6 - 1.4)
5	(0.7 - 13.1)	(1.6 - 10.3)	(3.2 - 7.2)	(3.7 - 6.5)	(4.2 - 5.9)
10	(3.2 - 20.7)	(4.8 - 17.2)	(7.4 - 13.0)	(8.1 - 12.1)	(8.8 - 11.3)
20	(9.5 - 34.3)	(12.2 - 29.7)	(16.3 - 24.1)	(17.3 - 22.9)	(18.3 - 21.8)

Cohorts of children with different ARV exposures may be compared to each other for the prevalence and incidence of abnormalities and other measures of morbidity and mortality. Groups will be formed based on therapeutic treatment regimen and comparisons made in the occurrence of HIV-related morbidity (e.g., lipodystrophy) and mortality. The power to detect two-and three- fold differences when comparing two groups is shown in Table 3 for study group sizes of between 50-500 subjects in each group, and baseline or reference rates of 1 to 15 percent.

Table 3. Power to detect a two-fold, and three-fold relative risk between two groups of equal sample size (2-sided alpha = 0.05).

Sample Size (in each group)	Reference Rate	Power to detect 2-fold Relative risk	Power to detect 3-fold Relative risk
50	1%	6%	11%
	5%	16%	38%
	10%	29%	71%
	15%	43%	92%
100	1%	8%	17%
	5%	27%	66%
	10%	51%	95%
	15%	72%	>99%
150	1%	11%	23%
	5%	38%	83%
	10%	68%	99%

	15%	88%	>99%
250	1%	15%	36%
	5%	56%	96%
	10%	88%	>99%
	15%	98%	>99%
500	1%	26%	62%
	5%	85%	>99%
	10%	99%	>99%
	15%	>99%	>99%

6.3 Analyses

Many analyses will focus on estimating the prevalence and incidence of clinical outcomes (e.g., opportunistic infections, growth failure), as well as the distribution of test results for continuous measures (e.g., viral load, CD4+ count). These estimates will be obtained for the total study group and for subgroups (e.g., infected children with undetectable viral load). Standard statistical parameters (e.g., mean, median, standard error, relative frequency, incidence rate) will be computed. When statistically practical, comparisons will be made between subgroups. Time-to-event analyses will include proportional hazards (PH) models that allow for heterogeneity/clustering in survival type data that are accruing from approximately 15-20 sites. Heterogeneity in survival data occurs because the application of treatment may vary by site. One analysis approach is a proportional hazards model that adjusts for clustering using a frailty (random effect) term (45,46). Sites or clusters would be the random effects in the proportional hazards model. The second approach is the marginal approach of Lin (47), which is also an extension of the PH model to handle multivariate failure time data. Survival curves (life table methods) will be constructed using the Kaplan-Meier (48) product limit method. Standard errors of the 1-5-year survival rates will be calculated using a modification to Greenwood's formula that stabilizes the tail of the survival distribution (49). These techniques are also appropriate for single group analyses to estimate key statistical parameters (e.g., age-adjusted incidence rate, 2-year survival). Poisson regression may be used for analysis of count data of rare events. Mixed effects models and generalized estimating equations or other regression methods will be used for continuous variables and for analyses of repeated measures collected longitudinally during the study. It is recognized that an association between an outcome measure, such as survival, and another variable, such as treatment, may arise due to confounding with other variables. Analyses will attempt to minimize such confounding by adjusting for important variables, such as virologic and immunologic parameters.

6.4 Statistical Monitoring and Interim Analyses

Several measures of accrual and follow-up will be monitored. These measures will be compiled at the individual site level as well as for all sites combined. They will be reported on a monthly basis, showing the history of accrual for each month and the cumulative total accrual. Other measures include enrollment by age, number of subjects off study and reason for discontinuation, and number and causes of death.

Individual participating investigators will be encouraged to submit data analysis concept sheets for additional analyses or sub studies of interest; these concepts will be reviewed by the PLACES Executive Committee in representation of the group.

Formal interim analyses will not be conducted. The Data Summary Report will be run and reviewed semiannually by Westat and NICHD. This report will contain summary statistics and frequency distributions of variables from the Case Report Forms, as well as derived or composite variables. The results will be stratified by country. Ad hoc data requests may also arise during the study. Statistical analyses will be conducted in response to concept sheets submitted by protocol team members.

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.

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. Laboratory performance reports will also be issued monthly indicating specimen collection and assay completion rates.

8.0 HUMAN SUBJECTS PROTECTION

8.1 Racial/Ethnic, Gender and Age Statement

Members of all racial and ethnic groups and both genders will be eligible for recruitment into this study. For the static cohort, the study will enroll eligible children who at the time of enrollment in the NISDI Pediatric Protocol had not yet attained their 6th birthday. For the dynamic cohort, the study will prospectively enroll eligible HIV-infected children prior to their 6th birthday. Strategies for recruitment will predominantly involve identification of currently (or newly) infected children followed at clinical sites. HIV-infected children identified during follow-up in the concurrent NISDI (LILAC) protocol of HIV-infected women and their exposed and uninfected infants and children at a funded clinical site, will be approached for enrollment into the present protocol.

8.2 Evaluation of Benefits and Risks/Discomforts

Infants who enter this protocol will have been infected *in utero* or intrapartum with HIV and will have received antiretroviral medications used for its treatment and prevention. Children who enter this protocol are those with established HIV infection. There have been several descriptive studies in the United States to assess, effects of potent combination ARV regimens and changes in the natural history of HIV infection in children. This will be one of the first long term studies of such outcomes in HIV infected children from Latin America. As a prospective observational cohort study, 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 these HIV-infected children had they not been enrolled in a study. 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. While there are no direct benefits to patients taking part in this study, information gained from their participation may lead to increased knowledge about the risk(s) of short and long term toxicity of antiretroviral treatment, and of HIV disease and its complications in this population.

8.3 Ethics Committee Review and Informed Consent

All participating sites 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 Harmonization (ICH)/Good Clinical Practices (GCP) guidelines. Should U.S. and in-country local/national regulations differ, the more restrictive guidelines will apply.

This is a prospective cohort study of not greater than minimal risk. As such, it does not require a Data and Safety Monitoring Board. All treatment(s) received by subjects enrolled in this observational study will be prescribed by clinicians caring for the enrolled individuals independent of this protocol. Since no protocol mandated intervention will be administered as part of this protocol, adverse events will not be reported.

This protocol, the informed consent and assent documents (Appendices VII and VIII), 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 parent or legal guardian of subjects. The minor subject's assent, must also be obtained if he or she is able to understand the nature, significance and risks associated with 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 parent or legal guardian.

Parent or legal guardian who is 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 parent or legal guardian'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

This study and a subject's participation may be discontinued at any time by the **primary caregiver's withdrawal of consent; the site's** local Ethics Committee or IRB; the country's national Ethics Committee or IRB; the country's Ministry of Health; or the NICHD.

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 NISDI protocol team. Any presentation, abstract, or manuscript will be made available for review by the protocol team members prior to submission. Principal Investigators (PIs) wishing to use NISDI pooled data and/or repository specimens for analyses should submit a written proposal. The concept sheet/outline should be submitted to the NISDI PLACES Protocol Executive Committee. Concept sheets regarding analyses addressing the primary objectives of the NISDI PLACES Protocol using study-wide, pooled data will be prioritized for publication or presentation at scientific meetings over ancillary submissions. Any presentation or submission for publication of NISDI data without prior NISDI PLACES Protocol Executive Committee approval is inconsistent with the spirit of collaborative research. Disregard of

this policy may result in a mandatory withdrawal of the abstract or publication and in an inability to participate in future NISDI publications.

10.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other bloodborne 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.

All specimens will be sent via regulations established by the International Air Transport Association. Please refer to individual carrier guidelines (e.g., FedEx, Airborne, or World Courier) for specific instructions.

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APPENDIX I

SCHEDULE OF EVALUATIONS – STATIC AND DYNAMIC COHORT

EVALUATIONS	Baseline	Visit Schedule: Chronological Age					
		6 months of age	1 years of age	1 ½ years of age	2 years of age	2 ½ years of age	Follow-up every 6 months
PRIOR TO ENROLLMENT							
Informed Consent	X						
Documentation of M**aternal** HIV Infection	X						
CLINICAL EVALUATIONS							
History	X	X	X	X	X	X	X
Physical Exam	X	X	X	X	X	X	X
Tricep Skinfold Thickness	X	X	X	X	X	X	X
Mid-upper Arm Circumference	X	X	X	X	X	X	X
Weight and Height	X	X	X	X	X	X	X
Head Circumference	At every visit until three years of age						
LABORATORY EVALUATIONS							
Complete Blood Count (CBC) with Differential and Platelets [1]	X (EDTA purple top) (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)
Biochemical Assays[2]	X (red-top - no additives) (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)
Fasting insulin[3]	**X (red SST) (3.5 ml)**						**X**
Fasting glucose[4]	**X (red SST) (1.0 ml)**						**X**
HIV RNA Viral Load Assays	X (2.0 ml)	X	X	X	X	X	X
Flow Cytometry (CD4+ and CD8+ absolute counts and percentages)[1]	X (from CBC 2.0 ml tube)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)
Stored Peripheral Blood Mononuclear Cells (PBMCs) and Plasma[5]	X (EDTA purple-top) **(**2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	X (2.0 ml)	Every 12 months (2.0 ml)
Maximum Blood Volume:	**12.5** ml	8.0 ml	**12.5** ml	8.0 ml	**12.5** ml	8.0 ml	**12.5** ml

FOOTNOTES TO SCHEDULE OF EVALUATIONS

[1] Lymphocyte subset collection will be from same 2.0 ml EDTA purple-top tube as the complete blood count with differential and platelets. Use Becton Dickinson tube No. 369651 or equivalent.

[2] Biochemical Assays: Use Becton Dickinson tube No. 369611 or equivalent. (Further reduction in required blood volume may be achieved with the use of a Becton Dickinson Microtainer red-top tube without additives (No. 365957) or equivalent with a volume of 800-900 microliters.)

All the participants will have the following collected: Aspartate aminotrasferase (AST [SGOT]), alanine aminotrasferase (ALT [SGPT]), total bilirubin, lactate dehydrogenase (LDH), BUN, creatinine, albumin, total protein, creatine phosphokinase [CPK], fasting cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), lipase and triglycerides.

[3] Fasting serum glucose will be obtained annually in all subjects 5 years of age and older.

[4] Insulin levels will be obtained annually in all subjects 5 years of age and older. If the calculated homeostatic model assessment insulin resistance (HOMA-IR=(fasting insulin x fasting glucose)/22.5) is greater than 2.5 in children (Tanner stage less than 2) Or greater than 4.0 in adolescents (Tanner stage greater than or equal to 2), then a 2-hour oral glucose tolerance test (OGTT) and an HbA1c will be recommended.

[5] Refer to Appendix V for guidelines regarding collection, processing, and transportation of these specimens. Use Becton Dickinson tube No. 369651 or equivalent.

APPENDIX II

DEFINITION OF HIV INFECTION STATUS

The protocol will utilize the following definitions of HIV infection status.

A. HIV-infected

For children <18 months old when tested, born to an HIV-infected mother and has positive results on two or more of the following (separate determinations) (excluding cord blood):

- Positive HIV culture;
- Positive HIV DNA PCR;
- Positive quantitative p24 antigen above assay cutoff; and
- Quantitative HIV RNA ≥ **10,000** copies/ml.

For children ≥ 18 months old when tested, born to an HIV-infected mother and has positive results on two or more of the following (separate determinations on separate blood specimens):

- Reactive test for HIV antibody with confirmatory test by Western Blot or Immunofluorescence assay.
- Positive HIV culture;
- Positive HIV DNA PCR;
- Positive quantitative p24 antigen above assay cutoff; and
- Quantitative HIV RNA ≥ 1,000 copies/ml.

APPENDIX III

CENTERS FOR DISEASE CONTROL AND PREVENTION CLASSIFICATION SYSTEM FOR HIV INFECTION IN CHILDREN LESS THAN 13 YEARS OF AGE

(Modified from *"1994 Revised Classification System For HIV-Infection In Children Less Than 13 Years Of Age"*, Morbidity and Mortality Weekly Report, 1994; 43 [RR-12])

NOTES:

1) For this protocol, the criteria in this Appendix will also be applied to subjects ≥ 13 years of age.
2) This system will be utilized by the protocol to provide a standardized classification system. This system is not intended to provide clinical care standards.
3) Please see MMWR, 1994; 43 [RR-12]:1-10 for full text, references and acknowledgments.

SUMMARY

This revised classification system for human immunodeficiency virus (HIV) infection in children replaces the pediatric HIV classification system published in 1987 (1). This revision was prompted by additional knowledge about the progression of HIV disease among children.

In the new system, infected children are classified into mutually exclusive categories according to three parameters: a) infection status, b) clinical status, and c) immunologic status. The revised classification system reflects the stage of the child's disease, establishes mutually exclusive classification categories, and balances simplicity and medical accuracy in the classification process. This document also describes revised pediatric definitions for two acquired immunodeficiency syndrome-defining conditions.

IMMUNOLOGIC CATEGORIES

The immunologic category classification (Table 2) is based on either the CD4+ T-lymphocyte count or the CD4+ percent of total lymphocytes. If both the CD4+ count and the CD4+ percent indicate different classification categories, the child should be classified into the more severe category. Repeated or follow-up CD4+ values that result in a change in classification should be confirmed by a second determination. Values thought to be in error should not be used. A child should not be reclassified to a less severe category regardless of subsequent CD4+ determinations.

CLINICAL CATEGORIES

Children infected with HIV or perinatally exposed to HIV may be classified into one of four mutually exclusive clinical categories based on signs, symptoms, or diagnoses related to HIV infection (Box 2). As with the immunologic categories, the clinical categories have been defined to provide a staging classification (e.g., the prognosis for children in the second category would be less favorable than for those in the first category).

Category N, not symptomatic, includes children with no signs or symptoms considered to be the result of HIV infection or with only one of the conditions listed in Category A, mildly symptomatic. Category N was separated from Category A partly because of the substantial amount of time that can elapse before a child manifests the signs or symptoms defined in Category B, moderately symptomatic. Also, more staging information can be obtained during this early stage of disease by separating Categories N and A. In addition, for children who have uncertain HIV-infection status (prefix E), Categories N and A may help to distinguish

those children who are more likely to be infected with HIV (23) (i.e., children in Category EA may be more likely to be infected than children in Category EN).

Category B includes all children with signs and symptoms thought to be caused by HIV infection but not specifically outlined under Category A or Category C, severely symptomatic. The conditions listed in Box 2 are examples only; any other HIV-related condition not included in Category A or C should be included in Category B. Anemia, thrombocytopenia, and lymphopenia have defined thresholds in the new classification system (23).

Category C includes all AIDS-defining conditions except lymphoid interstitial pneumonitis (LIP) (Box 3). Several reports indicate that the prognosis for children with LIP is substantially better than that for children who have other AIDS-defining conditions (21,24,25). Thus, LIP has been separated from the other AIDS-defining conditions in Category C and placed in Category B.

Signs and symptoms related to causes other than HIV infection (e.g., inflammatory or drug-related causes) should not be used to classify children. For example, a child with drug-related hepatitis or anemia should not be classified in Category B solely because these conditions may be associated with HIV infection. In contrast, a child with anemia or hepatitis should be classified in Category B when the condition is thought to be related to HIV infection. The criteria for diagnosing some conditions and determining whether a child's signs, symptoms, or diagnoses are related to HIV infection may not be clear in all cases, and therefore may require judgment of the clinicians and researchers using the classification system.

TABLE 1. Pediatric human immunodeficiency virus (HIV) classification*

Clinical Categories				
Immunologic Categories	N: No signs/ symptoms	A: Mild signs/ Symptoms	B:** Moderate signs/symptoms	C:** Severe signs/symptoms
1: No evidence of suppression	N1	A1	B1	C1
2: Evidence of moderate suppression	N2	A2	B2	C2
3: Severe suppression	N3	A3	B3	C3

* Children whose HIV infection status is not confirmed are classified by using the above grid with a letter E (for perinatally exposed) placed before the appropriate classification code (e.g., EN2).

TABLE 2. Immunologic categories based on age-specific CD4+ T-lymphocyte counts and percent of total lymphocytes

Immunologic Category	Age of Child		
	< 12 months	1-5 years	6-12 years
	l (%)	l (%)	l (%)
1: No evidence of suppression	≥ 1,500 (≥ 25)	≥ 1,000 (≥ 25)	≥ 500 (≥ 25)
2: Evidence of moderate suppression	750-1,499 (15-24)	500-999 (15-24)	200-499 (15-24)
3: Severe suppression	< 750 (< 15)	< 500 (< 15)	< 200 (< 15)

Box 2. Clinical Categories for Children with Human Immunodeficiency Virus (HIV) Infection

CATEGORY N: NOT SYMPTOMATIC

Children who have no signs or symptoms considered to be the result of HIV infection or who have only one of the conditions listed in Category A.

CATEGORY A: MILDLY SYMPTOMATIC

Children with two or more of the conditions listed below but none of the conditions listed in Categories B and C.

- Lymphadenopathy (≥ 0.5 cm at more than two sites; bilateral = one site)
- Hepatomegaly
- Splenomegaly
- Dermatitis
- Parotitis
- Recurrent or persistent upper respiratory infection, sinusitis, or otitis media

CATEGORY B: MODERATELY SYMPTOMATIC

Children who have symptomatic conditions other than those listed for Category A or C that are attributed to HIV infection. Examples of conditions in clinical Category B include but are not limited to:

- Anemia (< 8 gm/dL), neutropenia (< 1,000/mm^3), or thrombocytopenia (<Ê100,000/mm^3) persisting ≥ 30 days
- Bacterial meningitis, pneumonia, or sepsis (single episode)
- Candidiasis, oropharyngeal (thrush), persisting (> 2 months) in children > 6 months of age
- Cardiomyopathy
- Cytomegalovirus infection, with onset before 1 month of age
- Diarrhea, recurrent or chronic
- Hepatitis
- Herpes simplex virus (HSV) stomatitis, recurrent (more than two episodes within 1 year)
- HSV bronchitis, pneumonitis, or esophagitis with onset before 1 month of age
- Herpes zoster (shingles) involving at least two distinct episodes or more than one dermatome
- Leiomyosarcoma
- Lymphoid interstitial pneumonia (LIP) or pulmonary lymphoid hyperplasia complex
- Nephropathy
- Nocardiosis
- Persistent fever (lasting > 1 month)
- Toxoplasmosis, onset before 1 month of age
- Varicella, disseminated (complicated chickenpox)

CATEGORY C: SEVERELY SYMPTOMATIC

Children who have any condition listed in the 1987 surveillance case definition for acquired immunodeficiency syndrome (10), with the exception of LIP (Box 3).

BOX 3. Conditions included in clinical Category C for children infected with human immunodeficiency virus (HIV)

CATEGORY C: SEVERELY SYMPTOMATIC *

- Serious bacterial infections, multiple or recurrent (i.e., any combination of at least two culture-confirmed infections within a 2-year period), of the following types: septicemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ or body cavity (excluding otitis media, superficial skin or mucosal abscesses, and indwelling catheter-related infections)
- Candidiasis, esophageal or pulmonary (bronchi, trachea, lungs)
- Coccidioidomycosis, disseminated (at site other than or in addition to lungs or cervical or hilar lymph nodes)
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis or isosporiasis with diarrhea persisting >1 month
- Cytomegalovirus disease with onset of symptoms at age >1 month (at a site other than liver, spleen, or lymph nodes)
- Encephalopathy (at least one of the following progressive findings present for at least 2 months in the absence of a concurrent illness other than HIV infection that could explain the findings): a) failure to attain or loss of developmental milestones or loss of intellectual ability, verified by standard developmental scale or neuropsychological tests; b) impaired brain growth or acquired microcephaly demonstrated by head circumference measurements or brain atrophy demonstrated by computerized tomography or magnetic resonance imaging (serial imaging is required for children <2 years of age); c)acquired symmetric motor deficit manifested by two or more of the following: paresis, pathologic reflexes, ataxia, or gait disturbance Herpes simplex virus infection causing a mucocutaneous ulcer that persists for>1 month; or bronchitis, pneumonitis, or esophagitis for any duration affecting a child >1 month of age
- Histoplasmosis, disseminated (at a site other than or in addition to lungs or cervical or hilar lymph nodes)
- Kaposi's sarcoma
- Lymphoma, primary, in brain
- Lymphoma, small, noncleaved cell (Burkitt's), or immunoblastic or large cell lymphoma of B-cell or unknown immunologic phenotype
- Mycobacterium tuberculosis, disseminated or extrapulmonary
- Mycobacterium, other species or unidentified species, disseminated (at a site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
- Mycobacterium avium complex or Mycobacterium kansasii, disseminated (at site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
- Pneumocystis carinii pneumonia
- Progressive multifocal leukoencephalopathy
- Salmonella (nontyphoid) septicemia, recurrent
- Toxoplasmosis of the brain with onset at >1 month of age

Wasting syndrome in the absence of a concurrent illness other than HIV infection that could explain the following findings: a) persistent weight loss >10% of baseline OR b) downward crossing of at least two of the following percentile lines on the weight-for-age chart (e.g., 95th, 75th, 50th, 25th, 5th) in a child ≥ 1 year of age OR c) <5th percentile on weight-for-height chart on two consecutive measurements, ≥ 30 days apart PLUS a) chronic diarrhea (i.e., at least two loose stools per day for >30 days) OR b) documented fever (for ≥ 30 days, intermittent or constant)

APPENDIX IV

WORLD HEALTH ORGANIZTION HIV CLASSIFIFCATION - CHILD

WHO CASE DEFINITIONS OF HIV FOR SURVEILLANCE AND REVISED CLINICAL STAGING AND IMMUNOLOGICAL CLASSIFICATION OF HIV-RELATED DISEASE IN CHILDREN

This document outlines recent revisions made by the World Health Organization (WHO) to case definitions for reporting of HIV and the clinical and the immunological classifications of HIV-related disease. HIV case definitions are defined and harmonized with clinical staging and immunological classifications to facilitate improved HIV-related reporting, and better track the incidence, prevalence, and treatment burden of HIV infection, and appropriate planning of public health responses. The revised clinical staging and immunological classification of HIV are designed to assist in clinical management of HIV, particularly where there is limited laboratory capacity. The final revisions outlined here are derived from a series of regional consultations with member states in all WHO regions held throughout 2004-2005, comments from public consultation, and deliberations of a global consensus meeting held in April 2006.

Simplified HIV case definitions for reporting are provided, based upon laboratory criteria combined with clinical or immunological criteria. The clinical staging of HIV-related disease for adults and children and the simplified immunological classification is harmonized to a universal 4 stage system that includes simplified standardized descriptors of clinical staging events. The revised HIV case definitions and the clinical and immunological classification system proposed are intended for conducting public health surveillance and for use in clinical care services.

The proposed immunological classification outlines four bands of HIV-related immunodeficiency: no significant immunodeficiency, mild immunodeficiency, advanced immunodeficiency, and severe immunodeficiency.

WHO IMMUNOLOGICAL CLASSIFICATION FOR ESTABLISHED HIV INFECTION

	Age-related CD4 values			
HIV-associated immunodeficiency	**< 11 mo (%CD4+)**	**12 - 35 mo (%CD4+)**	**36-59 mo (%CD4+)**	**≥ 5 yrs (absolute no/mm^3 or %CD4+)**
None/not significant	> 35	> 30	> 25	> 500
Mild	30 - 35	25 - 30	20 - 25	350−499
Advanced	25 - 29	20−24	15−19	200−349
Severe	<25	<20	<15	<200 *or* <15%

The clinical events used to categorize HIV disease among infants, children, adolescents or adults living with HIV are divided into those for which a presumptive clinical diagnosis may be made (where syndromes or conditions may be diagnosed clinically or with basic ancillary investigations) and those requiring a definitive diagnosis (generally conditions described according to causation requiring more complex or sophisticated laboratory confirmation. The next table provides the specific clinical events and the criteria for recognizing them.

WHO PRESUMPTIVE AND DEFINITIVE CRITERIA FOR RECOGNIZING HIV RELATED CLINICAL EVENTS IN HIV INFECTED CHILDREN (LESS THAN 15 YEARS OLD)

(For use in children aged less than 15 years with confirmed HIV infection.)

CLINICAL EVENT	CLINICAL DIAGNOSIS	DEFINITIVE DIAGNOSIS
Clinical Stage 1		
Asymptomatic	No HIV related symptoms reported and no signs on examination.	Clinical diagnosis
Persistent generalized lymphadenopathy (PGL)	Swollen or enlarged lymph nodes >1 cm at two or more non-contiguous sites, without known cause.	Clinical diagnosis
Clinical Stage 2		
Unexplained persistent hepatosplenomegaly	Enlarged liver and spleen without obvious cause.	Clinical diagnosis
Papular pruritic eruptions	Papular pruritic vesicular lesions.	Clinical diagnosis
Extensive wart virus infection	Characteristic warty skin lesions; small fleshy grainy bumps, often rough, flat on sole of feet (plantar warts); facial, more than 5% of body area or disfiguring.	Clinical diagnosis
Extensive molluscum contagiosum infection	Characteristic skin lesions: small flesh-coloured, pearly or pink, dome-shaped or umbilicated growths may be inflamed or red; facial, more than 5% of body area or disfiguring. Giant molluscum may indicate more advanced immunodeficiency.	Clinical diagnosis
Fungal nail infections	Fungal paronychia (painful, red and swollen nail bed) or onycholysis (painless separation of the nail from the nail bed). Proximal white subungual onchomycosis is uncommon without immunodeficiency.	Clinical diagnosis
Recurrent oral ulcerations (two or more in six months)	Aphthous ulceration, typically with a halo of inflammation & yellow-grey pseudomembrane.	Clinical diagnosis
Unexplained persistent parotid enlargement	Asymptomatic bilateral swelling that may spontaneously resolve and recur, in absence of other known cause, usually painless	Clinical diagnosis
Lineal gingival Erythema (LGE)	Erythematous band that follows the contour of the free gingival line; may be associated with spontaneous bleeding.	Clinical diagnosis
Herpes zoster	Painful rash with fluid-filled blisters, dermatomal distribution, can be haemorrhagic on erythematous background, and can become large and confluent. Does not cross the midlines.	Clinical diagnosis
Recurrent upper respiratory tract infection (URTI)	Current event with at least one episode in past 6 months. Symptom complex; fever with unilateral	Clinical diagnosis

	face pain and nasal discharge (sinusitis) or painful swollen eardrum (otitis media), sore throat with productive cough (bronchitis), sore throat (pharyngitis) and barking croup-like cough (LTB). Persistent or recurrent ear discharge.	
Clinical Stage 3		
Unexplained moderate malnutrition	Weight loss: low weight-for-age, up to −2 standard deviations (SDs), not explained by poor or inadequate feeding and or other infections, and not adequately responding to standard management.	Documented loss of body weight of –2SD, failure to gain weight on standard management and no other cause identified during investigation.
Unexplained persistent diarrhoea	Unexplained persistent (14 days or more) diarrhoea (loose or watery stool, three or more times daily), not responding to standard treatment.	Stools observed and documented as unformed. Culture and microscopy reveal no pathogens.
Unexplained persistent fever (>37.5 ^{0}C intermittent or constant, for longer than one month)	Reports of fever or night sweats for longer than one month, either intermittent or constant, with reported lack of response to antibiotics or antimalarials. No other obvious foci of disease reported or found on examination. Malaria must be excluded in malarious areas.	Documented fever of >37.5 ^{0}C with negative blood culture, negative malaria slide and normal or unchanged CXR, and no other obvious foci of disease.
Persistent oral candidiasis (after first 6-8 weeks of life)	Persistent or recurring creamy white to yellow soft small plaques which can be scraped off (pseudomembranous), or red patches on tongue, palate or lining of mouth, usually painful or tender (erythematous form).	Microscopy or culture.
Oral hairy leukoplakia	Fine small linear patches on lateral borders of tongue, generally bilaterally, which do not scrape off.	Clinical diagnosis
Acute necrotizing ulcerative gingivitis or stomatitis, or acute necrotizing ulcerative periodontitis	Severe pain, ulcerated gingival papillae, loosening of teeth, spontaneous bleeding, bad odour, and rapid loss of bone and/or soft tissue.	Clinical diagnosis
Lymph node TB	Non acute, painless "cold" enlargement of peripheral lymph nodes, localized to one region. Response to standard anti-TB treatment in one month.	Histology or fine needle aspirate for ZN stain. Culture.
Pulmonary TB	Nonspecific symptoms, e.g. chronic cough, fever, night sweats, anorexia and weight loss. In the older child also productive cough and haemoptysis. Abnormal CXR. History of contact with adult with smear positive PTB. No response to standard broad spectrum antibiotic treatment	Isolation of *M. tuberculosis* on sputum culture (together with compatible symptoms).
Severe recurrent bacterial pneumonia	Cough with fast breathing, chest in drawing, nasal flaring, wheezing, and grunting. Crackles or consolidation on auscultation. Responds to course of antibiotics. Current episode plus one or	Confirmed by isolation of bacteria from appropriate clinical specimens (induced sputum, BAL, lung aspirate).

	more in previous 6 months	
Symptomatic LIP	No presumptive clinical diagnosis.	CXR: bilateral reticulonodular interstitial pulmonary infiltrates present for more than two months with no response to antibiotic treatment and no other pathogen found. Oxygen saturation persistently <90%. May present with cor pulmonale and may have increased exercise-induced fatigue. Characteristic histology.
Chronic HIV-associated lung disease (including bronchiectasis)	History of cough productive of copious amounts of purulent sputum (bronchiectasis only), with or without clubbing, halitosis, and crepitations and/or wheezes on auscultation;	CXR may show honeycomb appearance (small cysts) and/or persistent areas of opacification and/or widespread lung destruction, with fibrosis and loss of volume.
Unexplained anaemia (<8g/dl), neutropenia (<0.5X 10 ^{9}/L^{3}) or chronic thrombocytopenia (<50 x 10 ^{9}/L^{3})	No presumptive clinical diagnosis.	Laboratory testing, not explained by other non-HIV conditions, not responding to standard therapy with haematinics, antimalarials or anthelmintics as outlined in IMCI.
Clinical Stage 4		
Unexplained severe wasting, stunting or severe malnutrition not adequately responding to standard therapy	Persistent weight loss not explained by poor or inadequate feeding, other infections and not adequately responding in two weeks to standard therapy. Characterized by: visible severe wasting of muscles, with or without oedema of both feet, and/or weight-for-height of –3 SDs, as defined by WHO IMCI guidelines.	Documented weight loss of >-3 SD +/- oedema
Pneumocystis pneumonia (PCP)	Dry cough, progressive difficulty in breathing, cyanosis, tachypnoea and fever; chest indrawing or stridor. (Severe or very severe pneumonia as in IMCI). Usually of rapid onset especially in infants under six months of age. Response to high-dose co-trimoxazole +/- prednisolone. CXR typical bilateral perihilar diffuse infiltrates	Cytology or immunofluorescent microscopy of induced sputum or bronchoalveolar lavage (BAL), or histology of lung tissue.
Recurrent severe bacterial infection, e.g. empyema, pyomyositis, bone or joint infection, meningitis but excluding pneumonia	Fever accompanied by specific symptoms or signs that localize infection. Responds to antibiotics. Current episode plus one or more in previous 6 months	Culture of appropriate clinical specimen.
Chronic herpes simplex infection; (orolabial or cutaneous of more than one month's duration or visceral at any site)	Severe and progressive painful orolabial, genital, or anorectal lesions caused by HSV infection present for more than one month.	Culture and/or histology
Oesophageal candidiasis (or candida of trachea, bronchi or lungs).	Difficulty in swallowing, or pain on swallowing (food and fluids). In young children, suspect particularly if oral candida observed and food refusal occurs and/or difficulties/crying when	Macroscopic appearance at endoscopy, microscopy of specimen from tissue or macroscopic appearance at bronchoscopy or histology.

	feeding.	
Extrapulmonary/disseminated TB	Systemic illness usually with prolonged fever, night sweats, weight loss. Clinical features of organs involved, e.g. sterile pyuria, pericarditis, ascites, pleural effusion, meningitis, arthritis, orchitis pericardial or abdominal.	Positive microscopy showing AFB or culture of Mycobacterium TB from blood or other relevant specimen except sputum or BAL. Biopsy and histology.
Kaposi's sarcoma	Typical appearance in skin or oropharynx of persistent, initially flat, patches with a pink or blood-bruise colour, skin lesions that usually develop into nodules.	Not required but may be confirmed by : - typical red-purple lesions seen on bronchoscopy or endoscopy; - dense masses in lymph nodes, viscera or lungs by palpation or radiology; - histology.
CMV retinitis or CMV infection affecting another organ, with onset at age over 1 month.	Retinitis only. CMV retinitis may be diagnosed by experienced clinicians: typical eye lesions on serial fundoscopic examination; discrete patches of retinal whitening with distinct borders, spreading centrifugally, often following blood vessels, associated with retinal vasculitis, haemorrhage and necrosis.	Definitive diagnosis required for other sites. Histology. CSF polymerase chain reaction (PCR).
CNS toxoplasmosis onset after age 1 month.	Fever, headache, focal neurological signs, convulsions. Usually responds within 10 days to specific therapy.	Computed tomography (CT) scan (or other neuroimaging) showing single/multiple lesions with mass effect/enhancing with contrast.
Extrapulmonary cryptococcosis (including meningitis)	Meningitis: usually sub acute, fever with increasing severe headache, meningism, confusion, behavioural changes that responds to cryptococcal therapy.	CSF microscopy (India ink or Gram stain), serum or CSF CRAG or culture.
HIV encephalopathy	At least one of the following, progressing over at least two months in the absence of another illness: - failure to attain, or loss of, developmental milestones, loss of intellectual ability; or - progressive impaired brain growth demonstrated by stagnation of head circumference; or - acquired symmetric motor deficit accompanied by two or more of the following: paresis, pathological reflexes, ataxia, gait disturbances.	Neuroimaging demonstrating atrophy and basal ganglia calcification and excluding other causes.
Disseminated mycosis (coccidiomycosis, histoplasmosis, penicilliosis)	No presumptive clinical diagnosis.	Histology: usually granuloma formation. Isolation: antigen detection from affected tissue; culture or microscopy from clinical specimen or blood culture.
Disseminated mycobacteriosis, other than TB	No presumptive clinical diagnosis.	Nonspecific clinical symptoms including progressive weight loss, fever, anaemia, night

		sweats, fatigue or diarrhoea; plus culture of atypical mycobacteria species from stool, blood, body fluid or other body tissue, excluding lung.
Chronic cryptosporidiosis	No presumptive clinical diagnosis.	Cysts identified on modified ZN microscopic examination of unformed stool.
Chronic Isospora	No presumptive clinical diagnosis.	Identification of Isospora
Cerebral or B cell non-Hodgkin lymphoma	No presumptive clinical diagnosis.	Diagnosed by CNS neuroimaging:; histology of relevant specimen
Progressive multi focal leukoencephalopathy (PML)	No presumptive clinical diagnosis.	Progressive neurological disorder (cognitive dysfunction, gait/speech disorder, visual loss, limb weakness and cranial nerve palsies) together with hypodense white matter lesions on neuro-imaging or positive polyomavirus JC (JCV) PCR on CSF.
Symptomatic HIV-associated nephropathy	No presumptive clinical diagnosis	Renal biopsy
Symptomatic HIV-associated cardiomyopathy	No presumptive clinical diagnosis	Cardiomegaly and evidence of poor left ventricular function confirmed by echocardiography

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 World Health Organization (WHO) publication: WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children at for full text, references and acknowledgments.

Available at: http://www.who.int/hiv/pub/guidelines/hivstaging/en/index.html

APPENDIX V

WEIGHT AND GROWTH MEASUREMENTS

This section describes the procedures for measuring weight, height, length, head circumference, mid-upper arm circumference and, triceps skinfold thickness.

All weight and growth measurements are taken three times by the same observer. All measurements are taken on the right side of the subject being measured. If measurements need to be taken on the left side due to abnormalities, be sure to record this on the case report form.

How to Measure Head Circumference:

An accurate head circumference measure is obtained with a flexible non-stretchable measuring tape. The tape should be approximately 1 cm wide and have 0.1 cm increments.

The tape should be positioned at the most prominent part of the back of the head (occiput) and just above the eyebrows (supraorbital ridges). The tape should be pulled snugly to compress the hair and underlying soft tissues. Any braids, barrettes, or other hair decorations that will interfere with the measurement must be removed. Record head circumference in centimeters to the nearest 0.1 cm.

Head circumference is required for each visit through 3 years of age.

How to Measure Length:

Length will be measured using a length board and in the recumbent position for infants younger than 24 months of age. The length board should have a fixed headpiece with a moveable footpiece which is perpendicular to the surface of the table that the length board is on. Length measurements should be obtained while the subject is dressed in light underclothing or a diaper. The subject's shoes must be removed. Hair ornaments should be removed from the top of the head.

The subject should be placed on his/her back in the center of the length board so that he/she is lying straight and his/her shoulders and buttocks are flat against the measuring surface. The subject's eyes should be looking straight up. Both legs should be fully extended and the toes should be pointing upward with feet flat against the foot piece. If the subject is < 24 months but is longer than the length of the board, the subject can stand up for the measurement.

It is important that both legs be fully extended for an accurate and reproducible length measurement. If only one of the subject’s legs is extended during the length measurement, the measurement may be unreliable and inaccurate. Correctly positioning the subject for a length measurement generally cannot be accomplished without two measurers. Length should be measured to the nearest 0.1 cm.

How to Measure Height:

Height should be measured using a calibrated, wall-mounted stadiometer for children 24 months or older. For best results, the subject is measured wearing a gown that allows the measurer to visualize the subject's body position. The subject stands with bare feet close together, body and legs straight, arms at sides, relaxed shoulders, and head, back, buttocks, and heels against the wall or shaft of the

stadiometer. Instruct the subject to look straight ahead and stand tall, keeping heels on the ground. Bring the headboard down to the top of the subject's head while at eye-to-eye level with the subject and record the height. Height should be measured to the nearest 0.1 cm.

How to Measure Weight:

Zero the scale prior to each measure. Use a calibrated scale and use the same scale as each visit whenever possible. Subjects should be weighed without shoes, diapers and clothing. Instruct the subject to stand with both feet centered on the scale with arms at the sides. The subject should not move or hold onto anything during the measurement. Allow the scale to stabilize and record the weight in the units provided by the scale in kg. For infants, use an electronic or beam scale with non-detachable weights. Weight should be measured to the nearest 0.1 kg.

ANTHROPOMETRIC MEASUREMENTS

Please see the protocol manual of procedures for additional information on training, certification, and equipment.

General Instructions

All measurements are taken on the right side of the child being measured. Whenever possible, measurement should be taken by a team of two measurers. One measurer takes the measurements while the other records. The measurer calls out the results to the recorder. The recorder repeats the results and then calls out the name of the next measurement. The measurer keeps the measuring instrument in place until the recorder repeats the number. The recorder checks the examinee's position during the procedure. Circumferences and skinfold measurements are to be done three times by the same observer(s).

How to Measure Mid-Upper Arm Circumference

Circumferences should be recorded with the zero end of the tape held by the left hand above the remaining part of the tape held by the right hand. The plane of the tape around the body part should be perpendicular to the long axis of the body part being measured. Care should be taken to ensure that the tape is touching the surface of the skin, but is not altering contours or compressing tissue. Maintenance of a perpendicular plane with the tape touching but not altering contours of the body can be challenging when measuring the obese individual and requires extra care on the part of the examiner.

To locate the midpoint, the child's elbow is flexed to 90 with the palm facing superiorly. The measurer stands above or behind the child and locates the lateral tip of the acromion by palpating laterally along the superior surface of the spinous process of the scapula. The tape is placed from the acromion process to the tip of the olecranon and the midpoint is marked. The arm is now repositioned to hang loosely at the side with the palm facing the thigh. The tape is passed around the arm from left to right and the free and fixed ends transferred. Ensuring that the tape is at the same level as the mid upper-arm mark, it is tightened so that it touches the skin all around the circumference but does not compress the tissue or alter the contour of the arm. The circumference is then read. Because the arm in cross-section is not an exact circle but rather oval, some difficulty may be met in ensuring that the

tape actually touches the skin on the medial side of the arm. To ensure that this is so, the middle finger of the left hand can be used to gently press the tape to the skin.

How to Measure Triceps (Arm) Skinfold Thickness

Lange calipers will be calibrated with standard metal blocks on the day of each exam. Calipers require calibrating to less than 1.5 mm (i.e., 1.0 mm or less) at each of the 10-, 20-, 30-, 40- and 50-mm test distances. Calipers that do not meet the standards must be removed from service and repaired. Please contact Westat for replacement calipers to use while your calipers are being repaired.

When making measurements, the fold of skin should be firmly grasped between the left thumb and forefinger (for right-handed observers) and then raised. The fold may be pinched and raised several times to make certain that no musculature is grasped. The skinfold is held firmly with the thumb and index finger, and the calipers are placed below the thumb and finger. The grip on the caliper is released completely, allowing the spring to compress the fold. With the fold held, the reading should be taken 3 seconds after caliper jaw pressure is released. The width of the skin that is enclosed between the fingers cannot be standardized, in its absolute size, for all the sites of the body. With a larger subcutaneous layer, a wider segment of the skin must be "pinched" in order to form a fold compared with those whose adipose tissue is poorly developed. The width of the skin should be minimal, still yielding a well-defined fold. The depth of the skinfold at which the calipers are placed on the fold also requires comment. The two sides of the fold are not likely to be parallel when the skin is lifted by one hand, being narrower near the crest and larger toward the base. When the calipers are placed at the base, the resulting measurement is too large. The correct distance from the crest of a true fold is obtained when the surfaces are approximately parallel to each other and to the contact surfaces of the calipers.

It is extremely important to measure skinfolds accurately. Even after extensive practice, it is possible to make errors due to slight misplacement of the calipers or misreading of the dial. To avoid such errors, the following procedure is recommended:

- Skinfolds should be lifted two or three times to determine the fold to be measured before placing the calipers.
- Avoid becoming overly anxious to put the calipers in place before determining what is really to be measured.

The calipers are placed below the thumb and index finger, and the dial is read. The calipers must be removed and the skinfold released between each measurement.

The level for the triceps skinfold is the same for the upper arm circumference, as marked with the felt pen. It is midway between the acromion and the olecranon with the arm is bent at a right angle, and measurement is made at the marked point. (Please see above) With arm dropped and hanging loosely, the skinfold is then raised from the underlying muscle fascia at this point with a sweeping motion of the fingers to the point at which the observer is holding the fold between the index finger and thumb. The skinfold caliper is then applied to the vertical fold.

APPENDIX VI

SPECIMEN COLLECTION, PROCESSING, STORAGE, AND TRANSPORTATION PROCEDURES

(REVISED 12 JUNE 2007)

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 **on the Westat-NICHD website at https://www.nichdclinicalstudies.org/index.html under the NISDI Clinical Project link.**

BLOOD COLLECTION AND STORAGE SUPPLIES

The following are specific laboratory supplies needed for 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 website: 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) 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) Label all specimens with the patient identification (PID) number and the date and time of collection.
3) Transport specimen at room temperature to the laboratory for processing.

SPECIMEN PROCESSING AND STORAGE:

EQUIPMENT/SUPPLIES/REAGENTS

1. Anticoagulated blood
2. Laminar flow hood (minimum class 2, type A biosafety hood).
3. Gloves (latex, vinyl, nitrile).
4. Lab coat or protective gown.
5. Centrifuge with horizontal rotor, capable of speeds up to 1800xg, and equipped with aerosol safe canisters.
6. Microcentrifuge tube for cell counting, 0.5 mL
7. Sterile pipettes, graduated and transfer.
8. Pipetting device.
9. Sterile plugged pipette tips.
10. Micropipettors of various volumes.
11. Sterile cryopreservation vials**:** 1.**8** to 2mL 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. Example: Sarstedt cat# 72.694.006 (flat), 72.693.005 (conical)
12. 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
13. Sterile conical centrifuge tubes, 15mL and 50mL.
14. Hemacytometer and microscope, or automated cell counter (i.e. flow cytometer or Coulter Counter)
15. -70 deg C freezer
16. 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.
17. Nalgene "Mr. Frosty" (Nalgene Cryo 1oC 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)
18. 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
19. 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.
20. Fetal bovine serum (FBS), heat-inactivated at 56o C for 30 minutes (mix larger volumes several times while inactivating).
21. Dimethyl sulfoxide (DMSO). 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
22. Freezing medium (cryoprotective medium): 90 percent fetal bovine serum + 10 percent DMSO; chill on ice or place in refrigerator at 4 deg C for at least 30 minutes. Freezing medium may be stored up to 1 week. (Experience from some ACTG laboratories has shown that larger volumes can be prepared, aliquoted and stored at –20 deg C for up to one year.)
23. 0.4 percent Trypan blue solution. Store at 18 to 250 C. If crystallization occurs, filter as needed. Observe manufacturer's outdate. Example: Sigma #T81540, 100 mL.
24. Isopropanol-100 percent (if using Mr. Frosty)

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.

<u>To separate plasma from PBMCs</u>:

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.

Remove the plasma (which should be aliquoted into labeled tubes and stored frozen per instructions below). Remove plasma carefully to avoid disturbing the cell layer. Transfer plasma to a sterile centrifuge tube. If multiple tubes of same anticoagulant were drawn at the same time point, plasma should be pooled before storage aliquots are prepared.

Plasma Processing:

1) Centrifuge the separated plasma again at 800 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 plasma freezer vials with the PID number, protocol, date and time of collection, and vial number (based on total number of vials obtained per subject, for example vial #1 of 4, #2 of 4, etc). Label should be placed on tube so that contents are visible.
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. Separate the peripheral blood mononuclear cells (PBMCs) using density gradient centrifugation. Follow manufacturer's instructions with respect to blood/ ratios and centrifugation time and speed. Centrifuge at room temperature with NO brake.

2. **Ficoll-Hypaque Overlay Method:**
   a.) After plasma is removed for storage, add a volume of sterile 1X PBS or HBSS equal to the volume of plasma removed. Mix gently and thoroughly. This step will decrease clumping of the cells during separation.
   b.) Optional: Add another volume of HBSS or 1X PBS equal to the total blood volume.
   c.) Carefully and slowly pipet blood-HBSS or blood-PBS on top of ficoll-hypaque solution in sterile 15 or 50 mL conical centrifuge tubes. Use 3mL Ficoll in 15mL tubes and 12mL Ficoll in 50mL tubes. Blood should be slowly overlaid while holding the tube at an angle. (Suggestion: gently allow mixture to flow down side of tube and pool on top of ficoll surface without breaking surface plane). Note: The ratio of ficoll to whole blood may vary according to manufacturer's recommendations and laboratory experience. For example, some manufacturers recommend 4 parts diluted blood to 3 parts ficoll reagent, however practical experience in some labs has shown good results using 3 parts blood to 1 part ficoll.)
   d.) Centrifuge tubes at room temperature for 30 minutes at 400 xg (or in accordance with the density gradient solution manufacturer's recommendations). Note: The centrifuge brake must be turned OFF for the separation to be clean and to maximize retrieval of the PBMCs.

3. After centrifugation, transfer the cloudy interface or buffy coat (PBMC layer) into appropriately labeled 50 mL sterile centrifuge tubes, by carefully aspirating the cells with a sterile transfer pipette. Avoid aspirating the density gradient media by maintaining the pipette tip above the cell layer and SLOWLY drawing the cells up into the pipette.

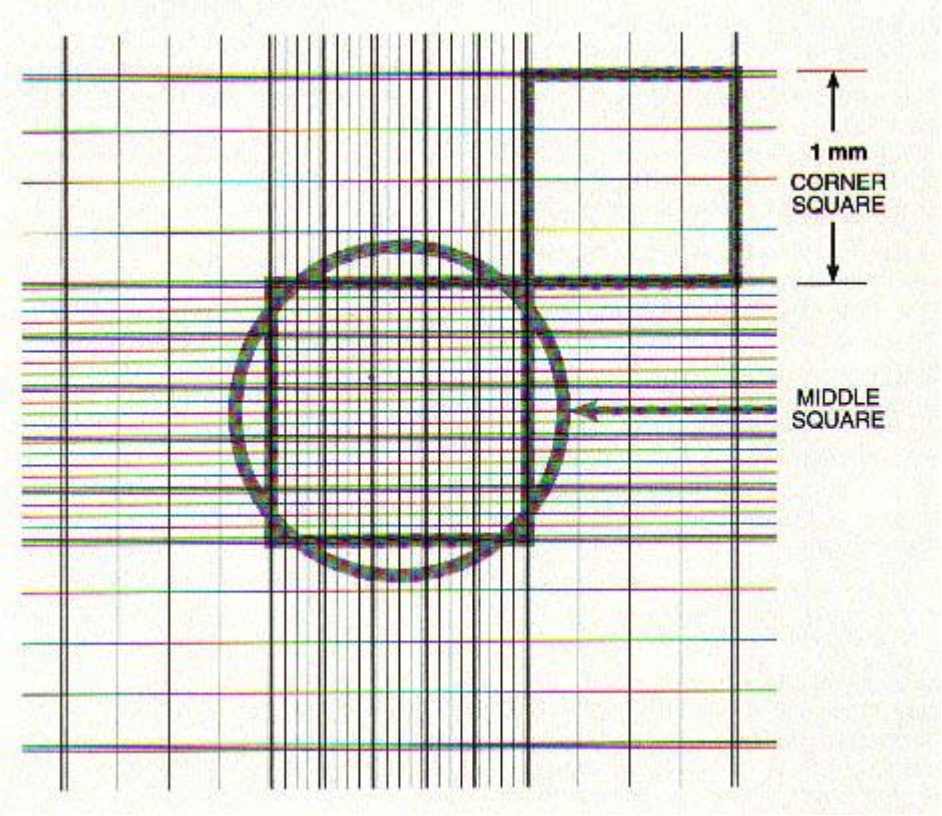
4. Wash PBMCs by diluting the PBMC layer with at least an equal volume of 1XPBS or HBSS. Centrifuge at 400xg for 10 minutes at room temperature. Aspirate and discard supernatant. A second wash is recommended.

5. Resuspend PBMC pellet in PBS, 30mL for a 50mL conical tube and 10mL for a 15mL conical tube.

Count and record the number of viable PBMCs per mL:

6. Pipette 10 uL of PBMC suspension into a 0.5 mL microcentrifuge tube. Add 90 uL of 0.4 percent Trypan Blue stain, making a 1:10 dilution (final concentration of Trypan Blue is 0.36%). Mix by pipetting thoroughly and carefully to avoid aerosol formation.
   i. Dilution Factor: $\frac{90\mu l \text{ Trypan Blue} + 10\mu l \text{ PBMC}}{10\ \mu l \text{ PBMC}} = 10^1$

7. Load the hemacytometer with cell mixture (Trypan Blue + PBMC's) until the area under the cover slip is sufficiently filled. Make sure to use a cover slip that is specific for the hemacytometer. Allow the cell suspension to settle in the hemacytometer for at least 10 seconds before counting. Count the four large corner squares. Count cells at 40x magnification. Viable PBMCs will be clear; nonviable PBMCs will be blue. NOTE: There should be 20 –100 cells per square. If the cell count falls outside of these brackets, repeat the count after the next wash increasing the volume in which the cells are resuspended if the cell number was higher than 100, or decreasing if the cell number was lower than 20.



8. Count cells in the 4 larger corner (1mm) squares. Include cells touching either the top line or left vertical perimeter line of any corner square. Do NOT count any cells which touch either the bottom line or right vertical perimeter line of any corner square.

9. **Calculate the number of PBMC/mL:**
   i. 10^4 = volume conversion factor to 1 mL

ii. 10^1 = specimen dilution factor

iii. PBMC/mL = $\frac{\text{PBMC in all four squares}}{4} \times 10^4 \times 10^1$

iv. example: $\frac{88}{4} \times 10^5 = 2.2 \times 10^6$ PBMC/mL

To calculate Cell Viability:

$$\text{Percent Viability} = \frac{\text{Number of Viable Cells Counted}}{\text{Total Number of Cells Counted}} \times 100$$

10. Automated counting may also be used. Follow manufacturer's instructions.

11. Freeze viable PBMC.

PBMC Freezing Procedure:

12. Label cryovials with the PID number, protocol, date and time of collection, specimen type and vial number (based on total number of vials obtained per subject, for example vial #1 of 4, #2 of 4, etc). Chill cryovials in the freezer for at least 10 minutes prior to cell freezing.

13. Prepare the freezing medium: 90 percent fetal bovine serum + 10 percent DMSO; chill on ice or place in refrigerator at 4 deg C. Freezing medium is good for two weeks if stored at 4 deg C.
14. Resuspend cells in cold freezing medium to achieve a concentration of 5-10 $x10^6$ cells/mL. Dispense 1.0 mL aliquots of the cell suspension into each cryovial. Be sure that the cryovial caps are securely tightened. Do not store more than 15 $x10^6$ cells/vial.

15. Immediately place the cryovials into in a slow-freeze container (e.g., "Mr. Frosty") which has been pre-chilled in the refrigerator (4 deg C). Place the "Mr. Frosty" containing cells in a -70 deg C freezer where it will be undisturbed for a minimum of 4 hours (up to 24 hours). Alternatively, place the cryovials into a controlled-rate LN2 freezing chamber (Cryomed Freezing Chamber or equivalent) that lowers the temperature 1 deg C per minute to -70 deg C.

NOTE:* *To prepare and use the "Mr. Frosty":
• Remove the high-density polyethylene vial holder and foam insert from the polycarbonate unit.
• Add 250mL of 100 percent isopropyl alcohol to the fill line. DO NOT OVERFILL. (Avoid slopping the isopropyl alcohol on the labels, causes ink to run.)
• Replace alcohol after every fifth use and document this reagent change.
• Carefully replace foam insert and vial holder.
• Place cryovials containing sample into holes in vial holder.
• Close "Mr. Frosty" and place in –70 deg C freezer.

16. After 4 to 24 hours in a –70 deg C freezer, transfer the cryovials into a LN2-rated box and place the box into vapor phase liquid nitrogen (-135 deg C) for long term storage. Avoid liquid phase storage due to safety concerns and to prevent possible problems with label adhesion failure.

NOTE: If cells are being frozen as nonviable PBMC Pellets (PEL), it is not recommended to resuspend cells in freezing medium because DMSO is a potent PCR inhibitor. If the PBMCs have been in contact with DMSO (e.g., freezing medium), wash the PEL 2x with PBS prior to storage**.**

Note 2: If there will be a delay in freezing the separated PBMCs, refrigerate the cells until ready to freeze. Centrifuge the cells at 200-400 xg at room temperature for 10 minutes. Aspirate off the complete medium from the pelleted cells. Then continue with PBMC Freezing Procedure section above for viable PBMCs.)

Note 3: If there is going to be a short delay, LESS THAN 15 MINUTES, in counting the cells after aspirating off the PBS or HBSS in section 6.1.2.9, and the PBMCs are intended for viable cryopreservation only, leave the pelleted cells in the tube at room temperature. The small amount of liquid that remains on the top of the PBMCs will prevent them from drying out. When ready to count the PBMCs, resuspend as described above.)

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 VII

SAMPLE INFORMED CONSENT

Full Title:
A PROSPECTIVE, OBSERVATIONAL STUDY
OF HIV-INFECTED CHILDREN AT CLINICAL SITES
IN LATIN AMERICAN COUNTRIES

Abbreviated Title:
NISDI Pediatric Latin American Countries Epidemiologic Study (PLACES)

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

INTRODUCTION

Your child is being asked to be in this research study because your child is infected with 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. The doctor in charge of this study at this site is: **(insert name of Principal Investigator)**. Before you decide if you want your child 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 have your child in this study, you will be asked to sign this consent form. Your child may also be provided with a simpler description of the study called an "assent." If your child is able to understand and agree to the study he or she may be asked to read and to sign the assent. You will get a copy of the consent (and assent) 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 children with HIV infection. These children will be tested to learn more about how the virus and the medicines they use to control the virus affect their health.

This study is called an observational study because health information is being looked at, or observed, by your doctor and other study staff. This information will be used to help understand the safety of medicines or other treatments that are used to prevent illnesses and treat HIV in children. Your child 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 your child if you chose for him or her not to be in this study. Since this is an observational study, we will be observing how your child's body handles the medicine, and what effects the medicines may have over time.

WHAT WILL MY CHILD HAVE TO DO AT THE STUDY VISITS?

If you choose to have your child in this observational study, your child will be given a patient identification number (PID). Any information collected will be recorded on study forms without your child's name, medical record number or other information which might tell people outside of the study who your child is.

Being in this study will involve both clinical evaluation and laboratory testing every six months for up to approximately five years. A standard physical examination will be done which will include measurements of his or her height and weight, and the thickness of the skin around the mid-upper arm. Head size and length will be measured on children less than 3 years old. Information about illnesses, prescribed medications and mother's medication history and pregnancy history (first visit only) will be also be gathered. If you are the child's mother we will ask for your permission to get this information from your medical record of the time when you were pregnant with your child.

WHAT TESTS WILL BE PERFORMED AT THE STUDY VISITS?

At each visit, blood tests will be performed to monitor your child's health (approximately 14 ml/3 teaspoons each visit). These tests will include studies of how well your child's kidneys, pancreas and liver function and the numbers of different types of blood cells. If your child is five years old or older, we will ask you to bring him or her to the clinic before eating breakfast (fasting), in order to do the blood tests that help to monitor how your child is handling the medicines used to treat his/her HIV. You will be asked to do this one time a year. We will monitor your child's immune system (how well the body fights infection) with CD4 cell counts and measure the amount of HIV in the blood (viral load).

In addition to the laboratory tests described above, your child's blood will be collected at every visit and stored for an unknown amount of time in a freezer for possible future scientific studies that may help answer new research questions related to HIV and HIV treatments. At no time will this extra blood be drawn if it is felt that it will harm your child's health in any way. These samples will be stored with your child's study ID (PID) number on them but not his/her name.

WHAT WILL BE DONE WITH MY (MY CHILD'S) STORED BLOOD SPECIMENS?

Your child's blood 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 medicines stop working (viral resistance); tests to measure the amount of anti-HIV medicine(s) in the blood; or new ways to know when HIV may cause symptoms of illness.

The future research studies to be done on your child'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. If the results of any study done on this stored blood may affect your child's health, the results will be given to the doctor in charge of the study at your site who will then give them to you. You may choose to withdraw your child's blood sample(s) from the storage freezer at any time. If you do not wish to allow the study team to store these samples, your child may still be in this study.

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

We expect that children will be in this study for a maximum of five years. Your child will have a study visit every six months during that time.

WHY WOULD THE DOCTOR TAKE MY CHILD OFF THIS STUDY EARLY?

The study doctor may need to take your child off the study early without your permission if:

- You or your child 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.

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 how HIV affects children and what the risks are for children taking anti-HIV medications for long periods of time.

WHAT OTHER CHOICES DOES MY CHILD HAVE BESIDES THIS STUDY?

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

WHAT ABOUT CONFIDENTIALITY?

Efforts will be made to keep both you and your child's medical records confidential (private), but complete confidentiality cannot be guaranteed. You (your child's) medical records may be opened if required by law. Results of tests performed with your (your child'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 (your child) will not be personally identified in any publication that results from the information collected in this study, since you (your child) will only be identified by an ID number. Individuals who might have access to your (your child'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 your (your child's) treatment outside of this study will be charged to you or your health insurance.

WILL I RECEIVE ANY PAYMENT?

You and your child will not receive any payment for being in this study.

Note: It is acceptable for clinical sites 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 CHILD IS) INJURED?

If your child is injured as a result of being in this study, the **(Insert name of the clinic)** will give your child 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 your child may receive additional treatment for injuries. There is no program for payment to you either through this institution or through 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 CHILD'S) RIGHTS AS A RESEARCH SUBJECT?

Taking part in this study is completely voluntary. You may choose not to take part (not to allow your child to take part) in this study at any time. You may take your child out of the study at any time. You and your child will be treated by this clinic the same way 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:
Insert
Name of the investigator or other study staff;
Telephone number of above.

For questions about your (your child's) rights as a research subject, contact:
Insert
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 my child out of this study at any time and I and my child will still have rights to receive medical care.

I am the child's biological mother and I agree to allow the study team to get information from my medical record about my pregnancy with this child

_____	_____	_____
Biological Mother's Name (Print or type)	**Biological Mother's Sign**ature*	Date

I give my permission to have my child take part in this study.

_____	_____	_____
Parent or Legal Guardian's Name (Print or type)	Parent or Legal Guardian's Signature	Date

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

Yes No (Circle one)

_____	_____	_____
Parent or Legal Guardian's Name (Print or type)	Parent or Legal Guardian's Signature*	Date
_____	_____	_____
Study Staff Conducting Consent Discussion (Print or type)	Study Staff Signature	Date
_____	_____	_____
Witness' Name (Print or type)	Witness' Signature	Date

APPENDIX VIII

SAMPLE ASSENT

Full Title:
A PROSPECTIVE, OBSERVATIONAL STUDY
OF HIV-INFECTED CHILDREN AT CLINICAL SITES
IN LATIN AMERICAN COUNTRIES

Abbreviated Title:
NISDI Pediatric Latin American Countries Epidemiologic Study (PLACES)

WHAT IS THIS STUDY ABOUT?

Acquired Immune Deficiency Syndrome, AIDS, is a disease that destroys the body's immune system (what the body uses to fight infection), so that a person can't fight germs well. The virus that causes AIDS is called the Human Immunodeficiency Virus, or HIV. You are being asked to be in a research study about children who have this infection. The reason we are doing this study is to try to understand how this virus affects your body and other kids like you. We will also study how medicines that you are taking for this virus work and how they may help heal the part of your body that fights against germs.

WHAT WILL HAPPEN TO ME IN THIS STUDY?

If you are in our study, a doctor or nurse will see you for a physical exam (they will listen to your heart, lungs and feel your belly). You will be weighed and measured to see how you are growing. This is the same type of exam you have always gotten at the clinic or doctor's office. You will not need to stay in the hospital for this study. The visits will all be in your doctor's office or at the clinic. Your parent(s) can be with you during the exam if you want. Your doctor or nurse will also ask you questions about how you are feeling. Please be sure to ask them any questions you may have.

When you come to clinic you will also need to get a blood test. These tests will help us know how well you are doing. You may be asked not to eat anything before you come to the clinic. The tests will tell us if the medicine(s) are helping your body fight your infection. As you know, it sometimes hurts a little when you get a blood test. Sometimes it can cause a bruise or even bleed a bit where the test was done. This will be the same kind of test as when the doctor draws your blood at regular doctor visits and should not hurt more.

WHAT IF I DON'T WANT TO BE IN THIS STUDY?

We have already talked with your parent(s) in order to get their permission for you to be in this study. Please talk about the study with your parent(s) or doctor before you decide to be in it. You can choose not to be in this study if you do not want to. You will still be able to see your doctor for regular clinic visits.

If you ever have any questions you can ask us at your doctor's visit or call us on the telephone. You can call Name of Investigator, coordinator, other research staff here.

If you want to be in the study, write your name on this page. We will give you your own copy of this paper after you sign it.

_____	_____	
Your Name	Date	
_____	_____	_____
Your Signature	Age	Grade
_____	_____	_____
Study Staff Conducting Assent Discussion (Print or type)	Study Staff Signature	Date
_____	_____	_____
Principal Investigator's Name (Print or type)	Principal Investigator's Signature	Date