PEDIATRIC PULMONARY AND CARDIOVASCULAR COMPLICATIONS OF VERTICALLY TRANSMITTED HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION

P^2C^2 HIV

PROTOCOL

June 14, 1993
(Revised 6/10/93)

NIH/NHLBI

PROTOCOL REVISIONS
(11/10/94)

Page	Section	Change
43	Table 13	Criteria for diagnosis of mycobacterial pulmonary infection
49	Figure 1	Reformated; no substantive change
50	Figure 2	Chronic Lung Disease Algorithm simplified

PROTOCOL REVISIONS
(11/10/93)

Page	Section	Change
vii		Table 20 revised; remove Table 21
66	Figure 4	Group IIb schema modified
69	Table 20	Group IIb schedule modified
70	Table 21	Deleted from Protocol

PROTOCOL REVISIONS
(09/01/93)

Page	Section	Change
6	2	Addition of sinusitis to Upper respiratory infection
34		Table 11 echocardiogram test schedule
35		Table 12

PROTOCOL REVISIONS
(06/10/93)

Page	Section	Change
i-vi		Table of Contents, Appendices, Tables
1		Estimates of transmission
3		Schedule of activities
22	4.1	Study design
25	4.2	Sample size
25	4.2.1	Randomization of Group IIb Cohort (NEW)
26	4.2.2	Estimation of Incidence or Prevalence (previously 4.2.1)
26a-28	4.2.3	Comparison of Complication Rates (previously 4.2.2)
30	4.4	Schedule of Routine Tests
31-38		Tables 8-12 Schedule of Routine Tests
40	5.1.2	HIV Testing of Mother and Infants
40	5.1.3	Immunologic/Virologic Assessment of Infants in Group I
41-42	5.1.4	Immunologic/Virologic Assessment of Mothers and Infants in Group II
45	5.2.2	DTPA studies discontinued
44		Table 14 Schedule of Pulmonary Studies
52	5.2.4.2	Frequency of Study (CXR)
56	5.2.3.5	DTPA studies discontinued
58	5.2.4.7	Lung Biopsy - Rationale (insert paragraph 2)
59	5.3.1	Fetal Echocardiography discontinued
64	5.3.2	Postnatal cardiac protocols
66		Figure 4
67-70		Tables 18-21
97	8.2.4	DTPA studies discontinued.
105	11.2.1	Clinical Centers (Principal Investigators updated)
106	11.2.2	Clinical Coordinating Center (Principal Investigator updated)
115-117	13	New Publication section
Appendix 1 Appendix 8		Apprendix 8 deleted *This appendix has been removed from the Protocol. Be sure to discard these test schedules used prior to 06/10/93.*

PROTOCOL REVISIONS
(09/01/92)

Page	Section	Changes
40	5.1.2	Addition of Polymerase Chain Reaction testing

PROTOCOL REVISIONS
(6/1/92)

Page	Section	Change
31	4.4.1	Second paragraph updated.
32	4.4.1	Accrual figures recalculated according to new recruitment schedule.
33	4.4.1	Wording added to indicate these are original assumptions. Footnote added.
34	4.4.1	Wording added to indicated these are original assumptions. Note added.
35 - 38	4.4.1	Wording added to indicate these are original estimates and to refer to Appendix 8 for current Protocol schedules.
41	5.1.4	Clarified wording on CMV and EBV testing in mothers *(First paragraph, 11 lines down)*
44	5.1.5	Table updated to reflect changes in PFT and DTPA testing.
45	5.2.2	Updated documentation for PFT and DTPA testing.
56	5.2.4.5	Reference to 5, 10 and 15 minutes removed *(See "Method").*
57	5.2.4.5	Updated documentation for DTPA testing *(See "Frequency of Testing").*
Appendix 8	-	Added.

NOTE: The update to section 5.2.4.4 (Pulmonary Function Testing) is pending additional information from the Pulmonary Subcommittee. The documentation will be updated upon receipt.

PROTOCOL REVISIONS
(4/1/92)

Page	Section	Change
64	5.3.2	Cardiac studies will be performed at six month intervals in Group IIb patients who do not have cardiac abnormlities. This group will not be randomized. The text to indicate randomization has been removed.
66	Figure 4	HIV will be determined at 18 months of age.
69	Table 20	Schedule changed. See above note for section 5.3.2.

NOTE: WE ARE AWAITING CHANGES FROM THE PULMONARY SUBCOMMITTEE TO UPDATE THE PULMONARY SECTION OF THE PROTOCOL (DTPA'S AND PFT'S). CHANGES WILL BE ADDED TO THE PROTOCOL UPON RECEIPT.

PROTOCOL REVISIONS
(9/25/91 & 10/1/91)

The following pages have been revised to reflect the Group I Eligibility Criteria revision:

Page 1

Page 22

Page 23

Page 24

Page 39

Page 40

Appendix 6

PROTOCOL REVISIONS
(05/01/91)

Page	Section	Change
58	5.2.4.6	Specimen Analysis
58	5.2.4.7	Lung Biopsy Processing and Analysis (Rationale and Methods)
77	5.3.6	Tissue Analysis
77	5.4	Pathological Studies: Postmortem...

PEDIATRIC PULMONARY AND CARDIOVASCULAR COMPLICATIONS OF VERTICALLY TRANSMITTED HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION

PROTOCOL

Table of Contents

Appendices:

Tables

Figures

ABBREVIATIONS

$A-aDO_2$	Alveolar arteriole oxygen difference
ABG	Arterial blood gas
ACTG	AIDS Clinical Trials Group
AG	Antigen
AIDS	Acquired immunodeficiency syndrome
ATS	American Thoracic Society
AZT	Azidothymidine
BAL	Bronchoalveolar lavage
BTPS	Body temperature and pressure, saturated
BX	Biopsy
CBC	Complete blood count
CC	Clinical Center
CCC	Clinical Coordinating Center
CD	Clusters of designation
CDC	Centers for Disease Control
CLD	Chronic lung disease
Cm	Centimeter
CMV	Cytomegalovirus
CNS	Central nervous system
CSF	Cerebral spinal fluid
CT	Computerized tomography
CX	Culture
CXR	Chest x-ray
DHST	Delayed hypersensitivity skin test
DHVD	Division of Heart and Vascular Diseases
DIP	Desquamative interstitial pneumonia
DLCO	Diffusing capacity for carbon monoxide
DLD	Division of Lung Disease
DNA	Deoxyribonucleic acid
DTPA	Diethylenetriaminepentacetate
EBNA	Epstein Barr Nuclear Antigen-1
EBV	Epstein-Barr virus
ECG	Electrocardiogram
EDD	End diastolic dimension
EDh	End diastolic wall thickness
ELISA	Enzyme linked immunosorbent assay
ESD	End systolic dimension
ESh	End systolic wall thickness
ESP	End systolic pressure
ESR	Erythrocyte sedimentation rate
ESS	End systolic stress
ESSc	End systolic stress - circumferential
ESSm	End systolic stress - meridional
FPI	Functional preload index
FRC	Functional residual capacity
FS	Fractional shortening
FWT	Fractional wall-thickening
HIV	Human immunodeficiency virus
HR	Heart rate
HSV	Herpes simplex virus
HX	History

Ig	Immunoglobulin
IL-2	Interleukin-2
IL-2r	Interleukin-2 receptor
IVDA	Intravenous drug abusers
IVL	Immunologic and Virologic Laboratory Studies
Kg	Kilogram
KS	Kaposi's sarcoma
LDH	Lactic dehydrogenase
LED	Light emitting diode
LIP	Lymphoid interstitial pneumonitis
LPLD	Lymphoproliferative lung disease
LV	Left ventricular
MAI	Mycobacterium avium-intracellulare
MAC	Mycobacterium avium-intracellulare complex
Mg	Milligram
Ml	Milliliter
NIAID	National Institute of Allergy and Infectious Diseases
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NHLBI	National Heart, Lung and Blood Institute
P^2C^2 HIV	Pediatric Pulmonary and Cardiac Complications of Vertically Transmitted Human Immunodeficiency Virus
p24Ag	Protein 24 Antigen
PA	Posterior Anterior
$PaCO_2$	Partial pressure - arterial carbon dioxide
PaO_2	Partial pressure - arterial oxygen
PCP	Pneumocystis carinii pneumonia
PCR	Polymerase chain reaction
PDSMB	The Policy, Data and Safety Monitoring Board
PEFV	Partial expiratory flow volume
PFT	Pulmonary function test
PLH	Pulmonary lymphoid hyperplasia
PPD	Purified protein derivative
PSS_m	Peak systolic meridional wall stress
Qst	Questionnaire
ROC	Receiver operating characteristics
RR	Respiratory rate
R-R	RR interval
RSV	Respiratory syncytial virus
SAS	Statistical Analysis System
SpO_2	Oxygen saturation from pulse oximetry
SSI	Stress-shortening index
SVI	Stress velocity index
Tc	Technetium
TMP-SMX	Trimethoprim-sulfamethoxazole
VCFc	Rate-adjusted mean velocity of shortening
Vmax	Maximum ventilation
VTI	Velocity time integral
WBC	White Blood Count
W.BLOT	Western Blot
WITS	The Women and Infant Transmission Study

Post-it™ brand fax transmittal memo 7671 | # of pages ▶ 1
To Dr Mark Schluchter | From H. Peavy
Co. Cleveland Clinic | Co. NHLBI
Dept. Bio Statistics | Phone # (216) 444-(301) 496-7732
Fax # (216) 445-6360 | Fax # (301) 496-9886

February 26, 1992

P2C2 HIV Schedules

	Original contracts		Policy Board Suggestion*		New Proposal 2/25/92 call	
Phases	yrs	dates	yrs	dates	yrs	dates
I Protocol development	1	5/22/89 to 5/21/90	1	5/22/89 to 5/21/90	1	5/22/89 to 5/21/90
II recruitment	2	5/22/90 to 5/21/92	3	5/22/90 to 5/21/93	2yrs 8mos	5/22/90 to 1/31/93
II follow-up	min 2.5 max 4.5	5/22/92 to 11/21/94	min 2 max 5	5/22/93 to 5/21/95	min 2 max 4yrs 8mos	2/1/93 to 1/31/95
III data analysis**	1	5/22/94 to 5/21/95	1	11/22/94 to 11/21/95	1	8/1/94 to 7/31/95

The new proposal is based on a review of the current budgets sent to you by Joanne Deshler. These indicate that we have enough money to extend the study by 2 months. The new schedule proposal that we discussed on 2/25/92 is shown above. It allows 2 years and 8 months for recruitment. The minimum time allowed for follow-up would be 2 years, which is the same as the schedule suggested by the Policy Board. The maximum follow-up would be 4 years and 8 months, which is actually slightly longer than the original contract schedule, but not quite as long as the 5 years allowed by the Policy Board's suggested schedule. If money is available in the last year of the study (e.g. less is spent than now projected) then follow-up should be extended to get as close as possible to the original minimum of 2.5 years.

* The Policy Board suggested that we extend recruitment for one year, but add only 6 months to follow-up (since it was clear that we did not have enough money to extend the study for a year). They thought that it was acceptable that the last subjects recruited could be followed for only 2 years.

** The schedule for data analysis does not change. It was always scheduled to be a one year period which overlaps with the last 6 months of recruitment.

1. Introduction

The overall objective of this program is to conduct a "natural history" study to characterize the pulmonary and cardiovascular disorders that occur in association with vertically transmitted (mother to child during gestation or during the perinatal time period) human immunodeficiency virus (HIV) infection in children. Although no interventions are planned in this study, it is recognized that treatment is likely to occur as the result of parallel studies in the same population of children. The study will be coordinated among five geographically separated centers (Baylor College of Medicine, Houston; Children's Hospital/Harvard Medical School, Boston; Mt. Sinai School of Medicine, New York City; Presbyterian Hospital/Columbia University, New York City; UCLA School of Medicine, Los Angeles and the Clinical Coordinating Center (The Cleveland Clinic Foundation) in the U.S.

Two groups will be studied. The groups are defined as follows:

Group I - Infants and children with vertically transmitted HIV-infection over 28 days of age.

Group II - Fetuses and infants of mothers infected with HIV.

Group I includes a cohort of children who are likely to develop extensive disease during the course of this study and therefore provides the opportunity to study the full range of cardiovascular and pulmonary complications that are associated with vertically transmitted HIV-infection in children. The Group II subjects provide the opportunity to determine the earliest features of infection in the fetus and longitudinally to follow these effects in the child. Since by current estimates 80 percent of Group II infants will not be infected, these uninfected infants will represent a control group for the 20 percent who are infected.

The primary objectives of the program are:

1. To collect information on the prenatal course and the subsequent pulmonary and cardiac structure, growth, and function of infants and children in Groups I and II.

2. To determine the types, incidence, course, and outcome of pulmonary and cardiovascular disorders in these children and to elucidate the etiology and pathophysiology of these lung and heart abnormalities.

Pulmonary disorders are almost universally present in children with acquired immunodeficiency syndrome (AIDS) many of whom are also thought to have heart abnormalities. The two most common pulmonary complications in pediatric AIDS patients are Pneumocystis carinii pneumonia (PCP) which is a major complication both in adults and children, and lymphoproliferative interstitial lung disease described as lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia (LIP/PLH complex) which is rarely seen with HIV-infection in adults. Pneumocystis carinii pneumonia and LIP/PLH complex may produce similar symptoms yet their prognoses appear to be different.

Pulmonary infections are the most commonly recognized life threatening disorders in patients with HIV-infection. Approximately one-half of the children who died of AIDS by 1985 were infected with Pneumocystis carinii. Although Pneumocystis carinii is the predominant pulmonary pathogen found in patients with HIV-infection, other organisms are of importance as well. The response of the immature lung to Pneumocystis carinii and other opportunistic lung infections complicating the course of HIV-infection is one major focus of this study.

The LIP/PLH complex is the other major pulmonary complication of AIDS in the pediatric age group. The etiology of this disorder, its pathogenesis, its relationship to the progression of the underlying HIV-infection, and its place in the spectrum of lymphoproliferative disorders of the lung that have been observed to complicate AIDS, are unclear. It is not clear whether LIP/PLH complex results from HIV-infection alone or is the manifestation of concurrent infection with HIV and other agents such as Epstein-Barr virus (EBV). HIV has been identified in cells lavaged from the lungs of children with LIP/PLH complex and in pulmonary alveolar macrophages.

Little is known about the effect on lung function of pulmonary abnormalities commonly complicating the course of AIDS in children, in part because it is difficult to perform serial lung function measurements on infants and young children. Adult patients with HIV-infection are found to have abnormalities in lung function such as decreased diffusing capacity for carbon monoxide (DLCO), lung volumes, and air flow. Whether tests of lung function, special radiologic techniques, or nuclear medicine studies may aid in early diagnosis of pulmonary disease in children with HIV-infection is not known.

Cardiac abnormalities have been demonstrated in adult patients with HIV-infection. These abnormalities have included left ventricular dysfunction, right ventricular dilatation, pericardial effusion, Kaposi's sarcoma and other tumors, non-bacterial thrombotic endocarditis, and electrocardiographic changes. Studies of the incidence and prevalence of cardiac abnormalities in the population of infants and children infected in utero and perinatally are limited despite the fact that this group is the largest and most rapidly growing population of children with HIV-infection. Recent studies on children with HIV-infection have documented the existence of electrocardiographic abnormalities, right and left ventricular dysfunction, and pericardial effusion. Very little is known of the etiology and pathophysiology of the heart dysfunction in this population, although there have been several reports of cytomegalovirus (CMV) being found in the heart in end-stage HIV-infection. It is known that a number of infectious agents are associated with heart muscle disease in patients with non-HIV disease. Many of these agents cause opportunistic infections in HIV-infected patients, but the extent to which they might be involved in heart damage in HIV-infected infants and children is unknown. Pediatric heart abnormalities associated with HIV infections appear to have a number of common electrocardiographic and echocardiographic findings and also show several features similar to the cardiac involvement seen in adults with HIV-infection. At the present time there has been no routine screening of children to document the prevalence of cardiac involvement in children with HIV-infection.

Although there are a few cases of congenital cardiac defects and myocardial dysfunction in infants exposed to maternal HIV infection, it is not clear whether those defects have their origins in developmental anomalies or result from the progressive effects of the HIV-related disease. A systematic study of the hearts of babies born with HIV-infection has not been done.

Because the total population of children in the United States with vertically transmitted HIV-infection is relatively small, it is expected that the subjects recruited into this study will also be participating in other epidemiologic and treatment studies. The study protocol for this program will not dictate treatment regimens, but will require that treatments be indicated on the data collection forms. The final protocol has taken this into consideration and is coordinated with ongoing studies of the mothers and children in this population.

The schedule of activities for 1989 to 1997 are summarized in Appendix 1.

2. Study Themes

The study goals, hypotheses and objectives are outlined in the following sections. The distinction between primary and secondary hypotheses and objectives is based upon the availability of information used to estimate parameters needed for sample size calculations. Scientifically, the secondary hypotheses and objectives are as important as the primary hypotheses.

2.1 Overall Goals

1. To determine the prevalence, incidence, and types of cardiovascular and pulmonary disease in the fetus, newborn, and child with vertically transmitted HIV-infection and to describe the course and outcome of these disorders.

2. To determine whether early detection of cardiovascular and pulmonary complications associated with vertically transmitted HIV-infection can be accomplished <u>in utero</u> by cardiologic methods and postnatally by sensitive cardiologic and pulmonary surveillance before they become clinically evident.

3. To determine whether immunologic dysfunction and/or co-infections (acquired before or after birth) in vertically transmitted HIV-infection lead to progressive pulmonary and/or cardiovascular disease.

2.2 Primary Hypotheses and Objective

Hypotheses

H1. The incidence of cardiovascular* complications is elevated in the population of fetuses exposed to maternal HIV infection, who subsequently as infants are proved to have HIV infection.

H2. The incidence of cardiovascular* and pulmonary** complications is elevated in the population of infants with vertically transmitted HIV-infection. The number of respiratory tract infections*** is also increased in this population.

Objective

O1. To estimate the frequency of specific pulmonary** and cardiovascular* complications in children symptomatic with HIV-infection.

*, **, *** See Page 6

2.3 Secondary Hypotheses and Objectives

Hypotheses

H3. The incidence of cardiovascular disease in HIV-infected children is increased with EBV and CMV infections.

H4. The incidence of lymphoproliferative interstitial lung disease in HIV-infected children is increased with EBV infection.

H5. HIV associated abnormalities of cardiovascular anatomy or function in the fetus persist into the neonatal period.

H6. Identifiable cofactors of HIV-infection are associated with alterations in cardiovascular anatomy and function in the neonatal infant and child and include:

a. Pulmonary/systemic bacterial/viral infection
b. Altered immune system
c. Primary treatment of HIV-infection
d. Perinatal events (including intrauterine growth retardation, drug exposure, or infection) and neonatal illness
e. Growth failure

H7. Identifiable cofactors of HIV-infection are associated with specific pulmonary** complications in the neonatal infant and child and include:

a. Altered immune system
b. Primary treatment of HIV-infection
c. Perinatal events (including intrauterine growth retardation, drug exposure, or infection) and neonatal illness
d. Growth failure

Objectives

O2. To determine whether pulmonary and cardiovascular disease associated with vertically transmitted HIV infection is detectable with sensitive diagnostic methods before clinical signs and symptoms appear.

O3. To estimate the recurrence rate of PCP.

O4. To determine whether PCP accelerates the rate of decline of lung function and shortens the life span of HIV-infected children with chronic lung disease.

* Specific cardiovascular complications related to these hypotheses for the fetus are a-h, and for the postnatal infant and child are b-i:

a. Altered umbilical blood flow profile
b. Structural heart disease
c. Altered myocardial performance
d. Pericardial effusion with and without hydrops
e. Arrhythmias
f. Altered chamber size and wall thickness
g. Cardiac valvular insufficiency or obstruction
h. Cardiac tumors or masses
i. Endocarditis (active or healed)

** Specific pulmonary complications related to these hypotheses are:

a. Upper respiratory infection (rhinitis, pharyngitis, otitis, sinusitis)
b. Fungal pulmonary infection
c. Viral pulmonary infection
d. Bacterial pulmonary infection
e. Mycobacterial pulmonary infection
f. Pneumocystis carinii pneumonia
g. Lymphoproliferative lung disease
h. Other interstitial lung diseases
i. Chronic obstructive lung disease
j. Airway hyper-reactivity
k. Upper airway obstruction
l. Pulmonary vascular disease

***Respiratory tract infections are a-f above, under specific pulmonary complications.

3. Disease Background

3.1 Natural History of Pediatric HIV Infection

Looking back just eight years and reviewing the explosive growth of pediatric human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) is a sobering and chilling experience. Between 1982 and 1985 when the first reports of a new lethal disease affecting the immune system of infants and children appeared (Oleske et al, 1983; Rubinstein et al, 1983; Scott et al, 1984; Cowan et al, 1984) and continuing up to the present when over 1700 cases of confirmed pediatric AIDS have been documented in the United States (Thomas et al, 1984; Rogers et al, 1987; Rubinstein and Bernstein, 1986; Falloon et al, 1989), it is extraordinary what havoc this new pediatric infectious disease has created in children and what morbidity and mortality it holds for children in the future. When one considers the projected total number of 3,300 cases of pediatric AIDS in the United States by 1992 (Rogers, 1988) and possibly 5 to 8 times that number of cases of pediatric HIV infection, it is imperative to regard pediatric HIV infection and AIDS as a major health threat for children, possibly the most important disease in pediatrics for the decade of the 1990's. Although the natural history of HIV infection in children as it affects the heart and lungs is not fully known, it is clear that secondary or opportunistic infections and unusual malignancies contribute significantly to the pronounced cardiac and pulmonary morbidity and mortality experienced by these children. Thus, EBV, CMV, opportunistic parasites, bacteria, fungi, viruses, and abnormal lymphoproliferative growths all produce pathological effects upon the heart and/or lung. These secondary agents cause the greatest morbidity and mortality in HIV-infected children.

3.1.1 Epstein-Barr Virus

One of the frequent complications of HIV infection is the occurrence of several types of pulmonary diseases in children, particularly LIP and PLH. (Shannon and Ammann, 1985). These unusual proliferations of lymphocytes, plasma cells, and immunoblasts are invariably associated with EBV (Andiman et al, 1985). As originally reported, LIP was found in young children less than two years of age (Joshi et al, 1985). Lymph node biopsies in LIP patients show follicular hyperplasia. These lesions probably represent the interaction of various viral, bacterial, and other agents with a defective immune system. In a longitudinal study, LIP was identified in patients with either normal proliferative responses to HIV antigens or abnormal responses (Blanche et al, 1986).

Epstein-Barr virus has been found in a lung biopsy of a child with LIP (Fackler et al, 1985). Interestingly this patient had no serologic response to EBV until 14 months of age. In a series of lung biopsies from children with AIDS and LIP, eight out of ten contained EBV DNA (Andiman et al, 1985). Most lacked antibody to the nuclear antigen of EBV despite having elevated titers to the early antigens. Epstein-Barr virus was found in saliva before DNA hybridization studies showed viral DNA in peripheral blood. Pulmonary lymphoid hyperplasia tends to present in older children (usually over two years of age) and is associated with parotid gland enlargement (Joshi and Oleske, 1986). In one study, ten out of eleven patients with PLH had elevated serum IgG levels, and four out

of five tested had EBV DNA in the tissue (Rubinstein et al, 1986). In PLH peribronchiolar nodular lymphoid aggregates as well as parenchymal lymphoid nodules are found. It has been suggested that PLH and LIP are a continuum (Joshi and Oleske, 1986). Lymphoid infiltrates have been found in the lungs, spleen, kidneys, liver, lymph nodes, colon, muscle, adrenal, thymus, and epicardium of patients who died with PLH (Joshi, Kauffman et al, 1987). Both LIP and PLH are reminiscent of those lymphoproliferative disorders seen in transplant patients having excessive immunosuppression (Shapiro et al, 1988).

The cardiac lesions of HIV-infected children are less well characterized than the pulmonary problems. Cardiac abnormalities have been reported in 25% to 73% of HIV-infected adults (Reitano et al, 1984). A dilated cardiomyopathy has been reported in children (Joshi et al, 1988). Myopathic abnormalities and hypertrophy are described with only rare foci of inflammatory infiltrates. Two of the patients described by Joshi had LIP and one had disseminated CMV infection. It is not clear what role EBV and CMV play in causing the cardiac lesions observed in pediatric HIV infections. Although rare, EBV has been reported to cause myocarditis (Wink and Schmitz, 1980; Frishman et al, 1977).

3.1.2 Cytomegalovirus

Evidence of infection with CMV (antibody, viral excretion, viremia) occurs in greater than 90% of adults with AIDS and serious CMV infections are reported in virtually every organ system. Pneumonitis, retinitis, esophagitis, gastritis, colitis, hepatitis, cholangitis, encephalitis, and adrenal necrosis are all manifestations of CMV disease in adult AIDS patients (Jacobson and Mills, 1983). In reports to the Centers for Disease Control (CDC), disseminated CMV infection has occurred in 19% of children with AIDS (Rogers et al, 1987). Serious CMV infections in AIDS patients are related more to a depression in cellular immunity than to the lack of humoral immunity and, in fact, CMV may not act as an important co-factor in the pathogenesis of HIV infection (Rook, 1988). For the HIV-infected population, CMV co-infection contributes to the already abnormal immune function. In a group of homosexual men, abnormal CD4/CD8 lymphocyte ratios were found primarily in those infected with CMV (Baroldi et al, 1988). A study of CMV viremia in HIV infected patients showed that the CMV antigen content increased as the immune deficiency worsened (Guarner et al, 1987). Nearly 50% of AIDS patients who are autopsied have CMV infection of the central nervous system or other organs.

The syndromes of CMV infection in children have not been described as precisely as those for adults and it is not clear if CMV causes the same spectrum of disease in children as it does in adults with AIDS. Cytomegalovirus can cause a primary pneumonitis in children or be found in association with other pulmonary pathogens, especially Pneumocystis carinii (Vernon et al, 1988). Gastrointestinal infections due to CMV in AIDS children are observed. Unusual manifestations in children of CMV infections of the GI tract include pyloric obstruction and enterocolitis (Victoria et al, 1985; Lim et al, 1988). CMV retinitis also occurs in children but the frequency of this complication is unknown.

3.1.3 Opportunistic Infections

Historically, AIDS was reported following recognition of opportunistic infection, namely PCP, in previously healthy males (Gottlieb et al, 1981). Even after the discovery and description of HIV, opportunistic infections in both adults and children are still the major cause of morbidity and mortality in the HIV-infected population. The CDC surveillance definition of AIDS, although updated to include serologic and virologic detection of HIV infection, continues to incorporate identification of opportunistic infection or recurrent serious bacterial infections as vital to the AIDS diagnosis (Centers for Disease Control, 1985; Centers for Disease Control, 1987). A list of such infections either definitively or presumptively diagnosed, is included in Table 1. Of the 40,836 cases of AIDS (adults and children) reported to the CDC September 1987 to December 1988, 33% reported PCP, 14% esophageal candidiasis and 6% extrapulmonary tuberculosis, primarily Mycobacterium avium-intracellulare (MAI). Thus, over 50% of the cases reported with opportunistic infection (Centers for Disease Control, 1989). Pediatric AIDS in the United States currently comprises under 2 percent of all reported AIDS cases. The incidence of opportunistic infections in children generally reflects adults statistics with a predominance of recurrent serious bacterial infections noted in children (Ochs, 1987).

TABLE 1

Opportunistic Infections/Recurrent Serious Infections

Inherent to CDC Surveillance AIDS Definition

Pneumocystis carinii pneumonia*

Candida esophagitis*

CNS toxoplasmosis (>1 month of age)*

Disseminated mycobacterial disease (MAC, MT6)*

Disseminated cytomegalovirus (CMV retinitis/vision loss)*

Disseminated histoplasmosis

Isosporiasis (chronic diarrhea)

Cryptosporidiosis (intestinal)

Disseminated coccidioidomycosis

Cryptococcosis (extrapulmonary)

Salmonella septicemia (recurrent)

Recurrent serious bacterial infections (Strep., Staph., H. flu., etc.)

Disseminated/persistent herpes simplex (>1 month)

* **Diagnosis of AIDS may be made presumptively for patients with these infections and documented HIV infection.**

Pneumocystis Carinii Pneumonia

Before 1981, PCP in the U.S. was reported predominantly in children with primary immunodeficiency or secondary immunodeficient states, i.e. neoplastic disease (Perera et al, 1970). In 1988, 33% of all U.S. AIDS cases (adult and pediatric) reported at least one episode of PCP. Morbidity and mortality from PCP is high with progression to hypoxic respiratory failure and mechanical ventilation noted in 25% of adults (Maxfield et al, 1988) and in greater than 50% of children (Marolda et al, in press). The most common opportunistic infection reported in children with HIV infection is PCP and it appears to have an especially fulminant course in those less than 1 year of age (Rogers et al, 1987). In children with AIDS, PCP ranks second only to LIP as the most frequently encountered pulmonary disease. Pneumocystis carinii, identified as a protozoan because of its response to antiprotozoan therapy, also has qualities of a fungus (Anderson and Hughes, 1987). Neither the route of transmission nor habitat of Pneumocystis carinii is defined although vertical transmission is suspected in some cases. Pathologically, the organism appears to be primarily restricted to lung tissue (attached to alveolar epithelial type II cells) although in rare cases reticuloendothelial involvement, blood borne infections, skin infections, and otitis media have been reported (Rahimi, 1974). Organisms have been demonstrated in sputum, bronchial washings, tracheal aspirates, and lung biopsies of symptomatic patients. The clinical manifestations of PCP in children have been distinguished from other pulmonary disease, i.e. LIP, by severity of hypoxemia, higher alveolar - arterial oxygen gradients, A-aDO_2, elevated serum lactate dehydrogenase levels, rapidity of disease progression with tachypnea, fever, characteristic diffuse interstitial infiltrates on chest radiograph, and lack of digital clubbing (Rubinstein et al, 1986). In adults with HIV infection, PCP may have a more insidious onset with symptoms of fever averaging 7 weeks and cough or dyspnea averaging 3 weeks before diagnosis (Engleberg et al, 1984).

The prognosis for PCP infection has improved since 1985 and this is probably related to enhanced detection techniques and earlier therapeutic intervention (Brenner et al, 1987). Pneumocystis carinii has been detected in sputum in over 50% of adult patients with AIDS ultimately found to have PCP (Kovacs et al, 1988). Since children rarely produce sputum, the definitive diagnosis of PCP relies upon identification of the organism in bronchial washings, tracheal aspirates or lung biopsies. Bronchoscopy with bronchoalveolar lavage is the preferred pediatric diagnostic procedure for PCP, but open lung biopsy yields the most definitive results in children.

Mycobacterium Avium-Intracellular Complex (MAC)

Mycobacterium avium-intracellular complex causes disseminated infections in both adults and children with AIDS. Other non-tuberculous mycobacteria can cause infection in AIDS patients but approximately 95% of the isolates associated with disseminated infection are MAC. In adult series, disseminated MAC has occurred in up to 50% of AIDS patients based on premortem or necropsy cultures or histologic findings (Hawkins et al, 1986; Young et al, 1986; Young, 1988). The incidence of disseminated MAC disease in children with AIDS is not as well

established but, in reports to the CDC, 43 of 552 (7.8%) children 0–9 years of age with AIDS were found to have disseminated MAC (Horsburgh and Selik, 1989). Surveillance studies by CDC show children actually have the highest frequency of disseminated MAC for any HIV-infected age group. However, the number of cases of disseminated MAC is probably under reported (Horsburgh and Selik, 1989). Rates of disseminated MAC vary in different areas of the country with the highest rates observed in the East and West South Central, New England, West North Central, and Mountain regions (Horsburgh and Selik, 1989).

The pathogenesis of MAC infection is not clear. These organisms are distributed widely throughout the environment yet cause disseminated disease infrequently in immunocompetent individuals. Since the organisms are found in stool and can invade the gastrointestinal tract, an anorectal portal of entry has been suggested in homosexual patients with AIDS (Damsker and Bottone, 1985). Investigators at the CDC have proposed that a common exposure to food or water is more likely since the rates of disseminated MAC are similar for age and race as well as for homosexual versus non-homosexual AIDS patients (Horsburgh and Selik, 1989). In addition, MAC may be recovered from sputum, bronchoalveolar lavage, pleural fluid, or other respiratory secretions prior to documentation of disseminated infection.

The clinical manifestations of disseminated MAC in children with AIDS have not been described as well as for adult AIDS patients but presumably they are quite similar. Fever, malaise, weight loss, weakness, anorexia, and night sweats are frequently noted (Hawkins et al, 1986; Young et al, 1986; Young, 1988). Abdominal pain, malabsorption, diarrhea, hepatosplenomegaly, and elevated levels of alkaline phosphatase are additional manifestations. One patient with disseminated MAC and abdominal pain developed a ruptured appendix, which on histologic examination contained histiocytes within which were acid-fast bacilli (Patrick et al, 1987). Unusual manifestations of MAC infection include extrabiliary obstructive jaundice secondary to lymphadenopathy and endobronchial masses (Packer et al, 1988).

Fungal Infections

Children with AIDS develop frequent oral infections with Candida albicans (thrush) which generally respond to systemic therapy with ketoconazole. Candida esophagitis was found in 20% of pediatric AIDS patients in the CDC national surveillance report (Rogers et al, 1987). Dysphagia is a common symptom of esophagitis due to Candida, CMV, Herpes simplex, or any other pathogen causing esophagitis.

Disseminated histoplasmosis is a well recognized opportunistic infection in AIDS patients and occurs more commonly in central areas of the United States in which Histoplasma capsulatum is endemic (Wheat et al, 1985; Graybill, 1988; Johnson et al, 1988). However, patients who have previously traveled to these areas or are from endemic regions outside the United States also may develop reactivated disease. Even though patients in the five centers of this NHLBI contract lie outside the classical "histo-belt" of the country, disseminated histoplasmosis can be the presenting sign of HIV infection, as recently documented in two children (Shearer et al, unpublished observations). Symptoms

and signs of disseminated histoplasmosis include fever, cough, lymphadenopathy, splenomegaly, pulmonary interstitial infiltrates, skin involvement such as macules or papules, and gastrointestinal invasion. In addition, disseminated histoplasmosis can present as a fulminant sepsis including disseminated intravascular coagulation and adult respiratory distress syndrome. Histoplasmosis can be isolated from blood or bone marrow cultures in a high percentage of infected AIDS patients. Culture and stains from other body tissues such as lymph node, lung, or skin may also yield the diagnosis. Central nervous system involvement may lead to positive CSF cultures.

Systemic salmonellosis has been associated with disseminated histoplasmosis in adult AIDS patients presumably related to blockage of the reticuloendothelial system by the histoplasmosis organism (Wheat et al, 1987). This association should be kept in mind when salmonella is isolated from blood cultures of pediatric patients with AIDS since salmonellosis is encountered frequently in these children.

Recurrent Bacterial Infections

Bacteria are common pathogens in both modest and serious infection in the normal host. In immunocompromised patients, especially those with B lymphocyte or granulocyte defects, recurrent serious bacterial infections are frequently reported and contribute significantly to morbidity and mortality. In HIV infected children documentation of recurrent serious bacterial infections noted as meningitis, osteomyelitis, pneumonia, sepsis, abscesses involving body organs, and septic arthritis with documented pathogens (H. influenzae type B, S. pneumoniae, S. aureus, etc.) is a criterion for establishing the diagnosis of AIDS (Centers for Disease Control, 1985). The first reports of significant numbers of HIV infected individuals with recurrent bacterial infections were noted in the pediatric HIV-infected population. Predilection for such recurrent bacterial infections is presumed secondary to immunologic naivete, impaired functional type specific antibody synthesis (despite polyclonal gammopathy), and impaired opsonization or granulocyte function (Bernstein et al, 1985; Krasinski et al, 1988; Murphy et al, 1988). Also, recurrent serious bacterial infections (S. aureus and S. pneumonia) are reported in adults (Jacobson et al, 1988).

Krasinski et al (1988) reported a 37% incidence of documented bacterial infections in 71 HIV positive children. Pneumonia, urinary tract infections and wound infections were most commonly identified, and bacteremia was noted in 28% of all documented infections. In this cohort, the most commonly encountered pathogens were S. pneumoniae and Salmonella species. It is notable that serious infections contributed to morbidity but not to mortality in this cohort.

Herpes Simplex/Varicella Zoster

Rogers et al (1987) reported chronic herpes simplex virus (HSV) infections in 5% of children reported with AIDS to the CDC. HSV has been associated with severe herpetic gingivostomatitis in infants and in adults. Cutaneous manifestations have included chronic perianal ulcers, as well as tracheobronchial mucosa and gastrointestinal tract involvement (Prose et al, 1987). For the most

part, HSV has been implicated in mild to severe localized infections but there have been no reports of disseminated HSV in children. Chronic HSV persisting for greater than one month in HIV positive children or adults fulfills the criteria for the diagnosis of AIDS.

Varicella zoster infections also contribute substantially to morbidity in HIV infected individuals (Quinnan et al, 1984). In adults, it has been suggested that the onset of zoster infections in HIV positive individuals heralds HIV-induced progressive loss of cellular immunity (Friedman-Kien et al, 1986). Disseminated herpes zoster described in immunocompromised hosts has also been described in AIDS (Cohen et al, 1988).

3.1.4 Neoplastic Diseases Associated with Pediatric HIV Infection

In the past two years, pediatric oncologists have been seeing an ever increasing number of children with AIDS-related lymphomas. The risk of lymphoma in adults has been reported to be 3-4% (Longo, 1984). The frequency of lymphomas may continue to increase as the number of at-risk children increases and new children are infected. Most congenitally HIV-infected infants developing lymphomas do so within the first five years of life, and of these about half will be diagnosed by 7 months of age and are likely to be profoundly immunosuppressed (Auger et al, 1988).

Thirteen children with AIDS-associated lymphomas have been reported by two of the largest centers treating children with AIDS, giving a frequency of 3-4% among all HIV-positive children in 1989 (Ellaurie et al, 1989; Epstein et al 1988). Three of these children had lymphomas involving the lung. Most of these patients were congenitally infected with HIV and one was a hemophiliac. Other reports of one or two patients included eight congenitally infected children and six with hemophilia (Pahwa et al, 1986; Andiman et al, 1985; Rechavi et al, 1987; Kamani et al, 1988; Patton et al, 1988; Cocchi et al, 1988; Chilcote, Williams et al, 1989; Belman et al, 1988). There were no reports of malignancies in patients with transfusion-acquired AIDS other than in hemophiliacs.

Infection with EBV has been implicated in these lymphoproliferations since the viral DNA is found in these tumors as well as in endemic Burkitt's lymphoma (Frizzera et al, 1980; Frizzera et al, 1981; Joshi, Kauffman et al, 1987; Rosenblatt et al, 1987; Shapiro et al, 1988). Evidence for a linkage between the virus and tumorigenesis has been strengthened by studies which strongly suggest that a clonal proliferation of EBV has occurred in the original tumor cell (Raab and Flynn, 1986; Cleary et al, 1988). In AIDS patients, EBV infections are poorly controlled and the opportunity for an uncontrolled B cell proliferation is enhanced (Birx et al, 1986). EBV DNA has been found in these proliferative lesions. As with lymphoproliferations of iatrogenically immune suppressed patients these may be precursors to malignant lymphomas.

Most cases of lymphoma in children with AIDS present with fever, weight loss, diffuse adenopathy, hepatomegaly, jaundice, and abdominal distention. Some of these children also have the pulmonary lymphoproliferative diseases including LIP or PLH which are further evidence of B cell dysregulation (Birx et al, 1986).

Children with CNS lymphomas may present with delay or loss of developmental milestones, dementia, cranial nerve palsies, seizures, or hemiparesis (Epstein et al, 1988; Pahwa et al, 1986; Andiman et al, 1985; Cocchi et al, 1988). The first two symptoms overlap those seen in children with diffuse HIV infection of the brain and the AIDS dementia complex (Belman et al, 1988). Computed tomographic studies with intravenous contrast of the brain show hyperdense mass lesions which are usually multicentric or periventricular (Pahwa et al, 1986).

Kaposi's Sarcoma (KS), is rare in children and does not resemble the rapidly progressive and clinically malignant epidemic form of KS observed in adults with AIDS. A lymphoadenopathic form of KS has been described in many patients including two children (Marguart et al, 1987; Buck et al, 1983). In this form of the disease, proliferative KS cells in lymph nodes cause lymphatic obstruction leading to lymphedema.

3.2 Overview of the Pulmonary Complications of Pediatric HIV Infection

3.2.1 Pneumocystis Carinii Pneumonia

PCP is one of the most frequent opportunistic infections in infants and children with HIV infection (Rubinstein et al, 1986; Joshi et al, 1984; Joshi et al, 1985). Infected children present with acute onset of fever, dry cough, tachypnea and hypoxia (Rubinstein et al, 1986). Breath sounds are diminished, and wheezing and localized or generalized rales may be heard on auscultation. The chest radiograph reveals diffuse bilateral interstitial and alveolar infiltrates with air bronchograms. Serum lactatic dehydrogenase activity is increased in HIV infected patients with PCP, presumably secondary to uncontrolled B lymphocyte proliferation in response to infection with PCP.

The use of pulmonary function testing to differentiate between PCP and other causes of respiratory deterioration is controversial. The predominant pulmonary abnormalities in adults with AIDS result from opportunistic infections, most commonly PCP or from Kaposi's sarcoma (Stover et al, 1985). The LIP/PLH complex, common in pediatric AIDS patients, is unusual in adult patients. Therefore, most of the pulmonary function tests in adults attempt to distinguish PCP from other causes of pulmonary deterioration. Several reports have demonstrated an increase in flow rates and decrease in lung volumes in adults with PCP (Stover et al, 1985; Stover and Meduri, 1988; Hopewell and Luce, 1985; Curtis et al, 1986), suggesting that lung elastic recoil is increased, possibly due to interstitial disease or interference with surfactant synthesis. Similar studies of pulmonary function in pediatric patients with HIV infection have not been reported. Measurements of static lung compliance can be performed in infants and have been shown to be a more sensitive indicator of parenchymal disease in infants with cystic fibrosis than respiratory rate or measurements of distribution of ventilation (Tepper et al, 1987). Similarly, since infants with PCP often wheeze, measurements of gas flow may become abnormal early on. While many techniques for measuring expiratory flow are not applicable to this patient population, the maximum flow at functional residual capacity (Vmax FRC) can be determined from partial forced exhalation flow volume curves (Taussig et al, 1982; Tepper and Hiatt, 1988).

Measurements of gas exchange are generally abnormal in adults with PCP and currently represent the best tests for distinguishing PCP from other causes of pulmonary deterioration (Stover et al, 1985; Stover and Meduri, 1988; Hopewell and Luce, 1985; Murray et al, 1984). The A-aDO_2 is usually elevated and is a sensitive indicator of PCP. This is especially true if the elevation is noted during exercise. In general, the degree of elevation is predictive of overall mortality. The diffusing capacity for carbon monoxide (DLCO) is also decreased, however, the decrease is not directly related to the quantity of PCP organisms in the lung (Stover and Meduri, 1988) and does not correlate with the A-a gradient for oxygen during exercise. This suggests that factors other than impaired diffusion affect gas exchange. The most likely explanation for the abnormality in gas exchange is a severe mismatching of ventilation and perfusion in the lung. In the infant, overall pulmonary blood flow is increased relative to lung mass (Feltes and Hansen, 1986) so even small changes in matching of ventilation and perfusion may result in an increase in the A-a gradient for oxygen. If this is the case, then the A-a gradient for oxygen may be a very sensitive indicator of early PCP infection.

Gallium 67 scanning has been studied extensively in adults with HIV infection and PCP. While an abnormal scan is a very sensitive index of parenchymal lung disease, it is not specific for PCP (Coleman et al, 1984). It has been suggested that HIV infected patients with PCP also have an increased uptake of 99Tc DTPA aerosols. This test is more attractive than Gallium scans in infants because the dose of radiation is lower and a second visit is not required. The sensitivity of this test for PCP in children is not known nor is its ability to distinguish PCP from LIP/PLH.

The definitive diagnosis of PCP requires identification of the organism in samples obtained from the tracheobronchial tree. Induction of sputum by inhalation of hypertonic saline is of use in adults if organisms are identified in the specimen (Clement et al, 1988). This test requires considerable cooperation and presently is of little use in children. In one study of 29 children with HIV infection, Bronchoalveolar lavage (BAL) correctly identified all 14 with PCP (Bye, Bernstein et al, 1987).

In the past, the diagnosis of PCP almost always required a lung biopsy. While this could be performed as a transbronchial biopsy in adults, in children an open lung biopsy is almost always necessary. Because of the complexities of open lung biopsy, it is reserved for the patient with progressive pulmonary deterioration in whom BAL is nondiagnostic.

Both trimethoprim-sulfamethoxazole (TMP-SMX) and pentamidine isethionate are effective in treating PCP. Both have a high rate of adverse reactions. Because of the nephrotoxicity associated with pentamidine, TMP-SMX is the drug of choice in the face of renal insufficiency while pentamidine is the obvious choice in patients with a history of allergy to sulfa drugs.

3.2.2 Lymphoid Interstitial Pneumonia/Pulmonary Lymphoid Hyperplasia

The most frequent pulmonary complication in children with AIDS is chronic progressive interstitial lung disease (Rubinstein et al, 1986). Lung tissue from HIV infected children with LIP or PLH reveals a diffuse infiltration of lymphocytes throughout the interstitium, into the alveolar septum and scattered nodules of mononuclear cells >0.5 mm in diameter (Rubinstein et al, 1986; Joshi et al, 1984; Joshi et al, 1985). These mononuclear cells, which are lymphocytes, plasma cells with Russell Bodies, plasmacytoid lymphocytes and immunoblasts, appear to be mainly B cells (Joshi et al, 1984). In patients with LIP these cells are spread diffusely through the lung parenchyma. In those with PLH, the lymphoid tissue is concentrated primarily in the walls of the bronchi and bronchioles without involvement of the alveolar septa. Both PLH and LIP are unusual findings in normal children or in adults infected with HIV (Stover and Meduri, 1988). Recently however, adults of Haitian origin have been described with LIP (Oldham et al, 1989).

The etiology of the LIP/PLH complex is unknown. The histologic findings have led some investigators to postulate that LIP/PLH results from a local response to a generalized antigenic stimulus (Joshi et al, 1985). This type of immune mediated tissue damage has been well characterized in recent years (Bach and Sachs, 1987; Rabin, 1983; Tilney et al, 1987). Briefly the process involves the following steps:

1. Immune recognition of specific antigens by host lymphocytes in the proper context of antigen presenting cells result in activation of CD4+ (helper) and CD8+ (suppressor/cytotoxic) T-cells.

2. Increased production of interleukin-2 (IL-2) and other lymphokines (primarily by CD4+ cells), increased expression of IL-2 receptors (IL-2r) and other activation antigens, and secretion of soluble IL-2r. (Rubin et al, 1985; Nelson et al, 1986).

3. Once activated, cells undergo rapid proliferative expansion resulting in a marked increase in the number of effector cells responsible for tissue damage.

As discussed previously in Section 3.1.1, several investigators have focused on chronic infection with HIV or EBV as the cause of LIP/PLH (Rubinstein et al, 1986; Katz et al, 1986; Andiman et al, 1985).

The LIP/PLH complex presents insidiously with cough, digital clubbing, salivary gland enlargement, lymphadenopathy and a typical chest radiograph revealing nodular interstitial infiltrates with or without hilar adenopathy. In the absence of secondary infection, digital clubbing, parotid gland enlargement, and persistent cough with a normal auscultatory examination characterize the disease. Serum IgG levels are uniformly elevated in patients with LIP/PLH and a peripheral lymphocytosis may be present.

Until the recent description by Oldham et al (1989), adults with HIV infection rarely were recognized to develop LIP/PLH, so little information is available regarding pulmonary function tests. Older children with LIP without

HIV infection have a reduced lung compliance with normal expiratory flows (Church et al, 1981; O'Brodovich et al, 1980). Static lung compliance is a sensitive marker of parenchymal lung disease in infants with cystic fibrosis and in fact is more sensitive than changes in respiratory rate or indices of gas mixing (Tepper et al, 1987). Therefore, a measurement of static lung compliance might be an early marker for LIP in infants with HIV infection. PLH on the other hand will be expected to alter gas flows and should affect the Vmax FRC measured by partial forced exhalation.

Gallium lung scans and 99Tc DTPA uptake are both abnormal in patients with LIP/PLH. Whether or not these tests will be specific for these interstitial lung diseases is unknown. However, thin cut CT scans of the chest are also likely to detect abnormalities in patients with interstitial lung disease and may represent one methodology for following the progression of the disease (Lynch et al, In Press), it is unclear whether this is feasible in children utilizing standard equipment.

Bronchoalveolar lavage in children with LIP/PLH yields a fluid comprised of roughly 40% lymphocytes, the majority of which are T8 cells. Unfortunately, this T8 preponderance occurs in patients with HIV infection without pulmonary disease and in patients with PCP (Solal-Celigny et al, 1985).

Lung biopsy is often required to establish the diagnosis of LIP/PLH. In young children, open biopsy is usually required in order to obtain enough tissue to make a diagnosis.

Treatment of LIP/PLH with corticosteroids has resulted in clinical improvements in some patients, but corroboration of this with pulmonary function testing or other objective measurements has not been reported (Pahwa, 1986).

3.2.3 Desquamative Interstitial Pneumonia (DIP)

DIP also occurs in children with HIV infection (Joshi et al, 1985). It is characterized by intra-alveolar accumulations of mononuclear cells, cuboidal metaplasia of the alveolar lining epithelium and septal fibrosis without lymphoid hyperplasia. The lymphoid depletion seen in patients with DIP suggests that they are unable to mount an immune response and, as such, may represent one extreme of a continuum of abnormalities of immune function.

3.2.4 Mycobacterial Diseases

Disseminated infection with MAI occurs commonly in children and adults with HIV infection. Symptoms include fever, anorexia, malaise, weight loss and diarrhea with cramping abdominal pain (Gold, 1988; Pitchenik et al, 1988). While the organism has been cultured from lung biopsy specimens, specific symptoms referable to the lung are not well documented. No treatment regimen has been shown to achieve sustained remissions, eradicate the organism or prolong survival, and drug toxicity may produce worse symptoms than the disease itself (Pitchenik et al, 1988; Hawkins et al, 1988).

3.2.5 Fungal Infections

Thrush and candida esophagitis are almost universal among patients with AIDS (Pahwa, 1988; Gold, 1988; Rubinstein et al, 1988). While Candida albicans is often recovered in bronchoscopic washings, invasive pulmonary disease is difficult to prove because of frequent colonization of the upper respiratory tract. Supraglottitis and vocal cord infections with candida have been observed in an HIV-positive infant presenting with hoarseness and upper airway obstruction (Rubinstein et al, 1988; Bye, Palomba et al, 1987).

Other opportunistic fungal infections have also been observed in children with AIDS including necrotizing bronchitis due to Aspergillus (Pervez et al, 1985), cryptococcal pneumonia and histoplasmosis.

3.2.6 Bacterial Infections

Bacterial pneumonia occurs more frequently in children with AIDS than in adults with AIDS (Vernon et al, 1988) and in fact recurrent bacterial infections are one of the diagnostic criteria for AIDS in HIV-positive children (Rogers, 1985). The most common offenders are the encapsulated organisms such as Streptococcus pneumoniae, Hemophilus influenzae b and Staphylococcus aureus. As the immunodeficient state progresses, infection with Gram-negative organisms such as Escherichia coli, Klebsiella, and Pseudomonas aeruginosa may result in a severe necrotizing bronchopneumonia. Institution of an aggressive broad-spectrum antibiotic therapy is recommended when there is clinical suspicion of bacterial disease. In addition, chronic gammaglobulin administration is being considered for treatment of patients suffering repeated bacterial infection.

3.2.7 Viral Infections

Disseminated cytomegalovirus infection is found at autopsy in many AIDS patients (Joshi et al, 1984; Joshi et al, 1985). CMV pneumonitis is common and may progress to acute respiratory failure or may simply be diagnosed incidentally in patients with other forms of pulmonary disease (Vernon et al, 1988). Combined therapy with ganciclovir (1,3-dihydroxy-2-propoxymethylguanine) and intravenous immune globulin has been used in infants with AIDS and CMV pneumonia (Pahwa, 1988) with some success. Other commonly acquired viral respiratory infections of childhood have not been well studied, and the contribution of these viruses to the progressive deterioration in pulmonary function accompanying HIV infection is not known. Respiratory syncytial virus infection has been associated with respiratory compromise and high mortality in young patients with AIDS (Chandwani et al, 1987). Other respiratory viruses such as parainfluenza, influenza, RSV and adenovirus are known to cause severe sequelae in immunocompromised children or those with pre-existing pulmonary and cardiac disease.

3.3 Background of Cardiovascular Status of Patients with AIDS

Reported cardiac complications in adults with AIDS include pericardial disease, myocarditis, endocarditis, cardiomyopathy, and Kaposi's sarcoma (Anderson et al, 1988; Corallo et al, 1987; Cohen et al, 1986; Lewis et al, 1985; Niedt and Shinella, 1985; Welch et al, 1984; Fink et al, 1984; Silver et al, 1984). Cammarosono and Lewis (1985) described the cardiac lesions from autopsies in 41 patients with AIDS. They found four cases in whom Kaposi's sarcoma involved the heart, three with non-bacterial thrombotic endocarditis, three with fibrinous pericarditis, and one with myocarditis. They concluded that cardiac manifestations occur frequently and may be relatively quiescent clinically but may lead to death. Anderson et al (1988) found myocarditis in 52% (37 of 71 AIDS cases) at necropsy in AIDS patients. The etiology of the myocarditis could not be determined in the majority of these cases although the likelihood of an acute viral cause seemed probable. Anderson, et al speculated that the viral myocarditis could be due to CMV, Coxsackie B, or HIV itself. Immune suppression may increase the possibility of infection with known cardiotropic viruses (O'Connell et al, 1986). Infection with enteroviruses produces myocarditis and there is evidence that myocarditis and/or myocyte necrosis can be due to EBV or CMV (Acierno, 1989; Cohen et al, 1986).

Rare forms of myocarditis resulting from a direct effect of commonly found viruses such as CMV may be the result of altered cellular and humoral immunity in AIDS (Wink et al, 1980; Cohen et al, 1988). Direct evidence for HIV infection of myocardiocytes, is lacking at this time. In an AIDS patient with myocarditis acute and convalescent phase titers for Coxsackie B2 were elevated (Dittrich et al, 1988).

Protozoal, bacterial, fungal, and mycobacterial opportunistic pathogens were present in myocardial sections of about 20% of autopsied patients (Anderson et al, 1988). In the 71 patients reported by Anderson et al (1988), 45 died from pulmonary failure or pneumonia. In adults with AIDS, recurrent opportunistic infection may be present for over six months before a rapidly fatal cardiac illness becomes evident 4-8 weeks before death (Cohen et al, 1986). Right ventricular dilatation may occur with or without hypertrophy or pericardial effusion (Anderson et al, 1988). Isolated right ventricular dilatation correlated positively with pulmonary failure. Biventricular dilatation was positively associated with pericardial effusion, and left ventricular (LV) dilatation occurred only in the setting of biventricular dilatation. Myocarditis, present in all cases of biventricular dilatation, occurred more frequently in the left ventricle but was also found in the right ventricle and ventricular septum. Seven of 71 patients had significant myocardial atrophy. Other secondary causes of cardiac abnormalities which may complicate the clinical course include cardiotoxins, hypersensitivity reactions and dietary deficiencies including vitamin deficiency (Cohen et al, 1986).

The incidence and prevalence of cardiac abnormalities in infants and children infected in utero and perinatally are not well documented despite the fact that this group is the largest and most rapidly growing population of children with HIV infection. At the present time, there has been no routine screening of children with HIV infection to document the prevalence of cardiac involvement in these children. Moreover, very little is known of the etiology

and pathophysiology of the cardiovascular dysfunction in this population. In a study of 175 children with HIV antibody perinatally-acquired from seropositive mothers, five children (2.8% incidence) were born with documented structural morphologic congenital heart defects (Vogel et al, 1988). The expected incidence in the general population for the same is approximately 0.8%, live births. The study did not address issues of fetal wastage due to complex congenital heart disease, and fetal echocardiograms were not done; nor was evidence presented that these five children were infected with HIV.

Cardiac complications of infants and children with AIDS have been reported. Steinherz and Brochstein (1986) described an infant with congestive cardiac failure three months after the diagnosis of AIDS. The patient had cardiomegaly, electrocardiographic signs of left ventricular hypertrophy and repolarization abnormalities, and echocardiographic signs of left ventricular dysfunction. Stewart et al (1989) found that congestive cardiac failure develops in many children with HIV infection (7 of 38). Cardiac compromise or congestive heart failure may complicate dilated cardiomyopathy in children with acquired immunodeficiency syndrome (Joshi et al, 1988).

It is probable that chronic cardiac disease precedes congestive cardiac failure. This cardiac compromise can be detected by echocardiography and electrocardiography (Issenberg et al, 1985). Echocardiographic abnormalities are frequent and consist of pericardial effusion, left ventricular dilatation, prolonged right ventricular systolic time intervals, and right ventricular dilatation (Sherron et al, 1985; Issenberg et al, 1985). Right ventricular dysfunction may follow repeated pulmonary infection and usually occurs in association with left ventricular dysfunction (Sherron et al, 1985). Hyperdynamic left ventricular function, a common finding in pediatric HIV infection, is often associated with autonomic instability, and may be a marker for arrhythmia or sudden death (Lipshultz et al, 1987).

Although high grade atrial and ventricular arrhythmias may not be evident clinically, fibrosis of the bundle of His and atrio-ventricular node, lobulation of the bundle, as well as vascular and perivascular changes with or without coronary occlusion have been described (Bharati et al, 1989). These necropsy findings suggest that undetected arrhythmias may be unmasked with repeat Holter examinations.

4. Study Design

4.1 Study Population

The cohort of subjects to be studied includes two groups:

Group I is composed of infants and children with documented vertically transmitted HIV-infection (Table 2). Children in Group I must have been born after April 1, 1985, except where vertical transmission of HIV infection can be documented with reasonable medical certainty, and be more than 28 days old. Vertical transmission of HIV-infection is considered documented when a mother is shown to be positive for HIV or dead due to AIDS (See Section 5.1.1). These children must have no evidence of sexual abuse. HIV-infection must be documented by the current CDC classification (Table 3). The disease status (P-1 or P-2 category) will be documented at the time of enrollment. Children who have a malignancy at diagnosis (P-2 Subclass E) are not eligible for study since treatment of their malignancies will confound the study (Table 3).

Group II is composed of infants born to HIV positive mothers. These infants must be enrolled in the study during gestation or on or before 28 days postnatal. Children in Group II who are determined to be infected with HIV (See Section 5.1.1, 5.1.2, and Table 3) are designated as Group IIa. The children in Group II who are not infected with HIV are designated as Group IIb. Children in Group II are expected to be classified as IIa or IIb by approximately 6 months of age. A random sample of 200 of the Group IIb non-infected children will be selected as controls for follow-up beyond 6 months of age. To provide a more homogeneous testing schedule for each child, the change in routine testing between Groups IIa and IIb is scheduled at 9 months of age. In order to address the hypothesized increased incidence of cardiovascular complications in HIV-positive fetuses (Section 2.1, H1), the statistical design (Section 4.2.2) requires 80% of children in Group II to be accrued during gestation.

Table 2

Inclusion and Exclusion Criteria

Inclusion Criteria

Group I

1. Children with documented vertically transmitted HIV-infection over 28 days of age (See Table 3 "Summary of the Definition of the HIV Infection in Children" for patients who are under 18 months of age)
   - Children born on or after April 1, 1985, except where vertical transmission of HIV infection can be documented with reasonable medical certainty

Group II

1. Children born to HIV-positive mothers
   - Prenatal enrollments - Pregnant women with documented HIV infection
   - Postnatal enrollments - Children less than or equal to 28 days of age at the time of enrollment

Exclusion Criteria

Group I

1. Children for whom informed consent cannot be obtained
2. Children less than or equal to 28 days of age
3. Children with CDC classification P-2 subclass E
4. Children with evidence of sexual abuse

Group II

1. Children for whom informed consent cannot be obtained

Table 3

Classification System for Human Immunodeficiency Virus (HIV) Infection in Children Under 13 years of Age*

Summary of the Definition of the HIV Infection in Children

Infants and Children Under 18 Months of Age Exposed to Infected Mothers

1. Virus in blood or tissues, or
2. HIV antibody, and evidence of both cellular and humoral immune deficiency, and one or more categories in Class P-2, or
3. Symptoms meeting CDC case definition for Pediatric AIDS

Older Children Exposed to Infected Mothers

1. Virus in blood or tissues, or
2. HIV antibody, or
3. Symptoms meeting CDC case definition for AIDS

Summary of the Classification of HIV Infection in Children Under 13 Years of Age

Class P-0. Indeterminate Infection

Class P-1. Asymptomatic Infection

- Subclass A. Normal immune function
- Subclass B. Abnormal immune function
- Subclass C. Immune function not tested

Class P-2. Symptomatic Infection

- Subclass A. Nonspecific findings
- Subclass B. Progressive neurologic disease
- Subclass C. Lymphoid interstitial pneumonitis
- Subclass D. Secondary infectious diseases
  - Category D-1. Specified secondary infectious diseases listed in the CDC surveillance definition for AIDS
  - Category D-2. Recurrent serious bacterial infections
  - Category D-3. Other specified secondary infectious diseases
- Subclass E. Secondary cancers
  - Category E-1. Specified secondary cancers listed in the CDC surveillance definition for AIDS
  - Category E-2. Other cancers possible secondary to HIV-infection.
- Subclass F. Other diseases possibly due to HIV-infection

*From: Morbidity and Mortality Weekly Report, Vol. 36, No. 15, 1987, pages 225-236. Massachusetts Medical Society.

(NOTE: Refer to Appendix 6, CDC Classification System, for a detailed description of the above categories.)

4.2 Sample Size

An estimated 200 Group I and an estimated 600 Group II subjects are expected to be entered into the study. The actual sample size of the Group II population may exceed 600 since the objective of the study is to examine a population of infected children and the number to be recruited is dependent on the transmission rate of HIV infection from mother to child. The transmission rate is estimated to be around 20%. Therefore, it is expected that 120 Group IIa (HIV-positive) subjects will ultimately be diagnosed as positive. The remaining children recruited into the study will be classified as Group IIb (HIV-negative) subjects on the basis of negative HIV cultures at 6 months and confirmed serologically after passively acquired maternal antibodies are no longer present. It is estimated that there will be 480 Group IIb subjects, 200 of whom will be selected as controls for follow-up beyond the age of 6 months.

At the time the RFP was issued the transmission rate was thought to be 40%. At the conclusion of the protocol development the transmission rate appeared to be 30%. More recent findings indicate that the transmission rate is closer to 20%. The transmission rate utilized to determine HIV-positive status may continue to change as the HIV epidemic progresses and as findings of other scientific studies are reported in the literature.

Section 4.2.1 describes the randomization of Group IIb subjects. Section 4.2.2 gives the precision of estimating an incidence or prevalence for various sample sizes that may occur in the study for different groups or subgroups. Section 4.2.3 gives the sample sizes required to compare complication rates in HIV-infected and non-infected children (Groups IIa and IIb) for various complication rates in the two groups. These sample size estimates relate to the primary hypotheses and objectives.

4.2.1 Randomization of Group IIb Cohort

It is estimated that there will be 480 Group IIb subjects. Two hundred of these subjects will be randomized as controls and the remaining 280 subjects will be randomized off the study.

The unit of randomization is the family. Any family with one or more Group IIb children will be eligible for randomization. When there is more than one Group IIb child in a family, only the first child classified as Group IIb will be considered part of the control group. If the first IIb child is randomized out, then the second IIb child in the family is also out of the study. If the first Group IIb child is randomized into the control group, then the second Group IIb child in the family will also be studied, though his/her data will be flagged and will not be considered part of the randomized control group.

Group IIb children who have siblings who are in Group IIa or Group I will be eligible for randomization using the method described below.

Group II children who are lost to follow-up or who die before their HIV status is known will not be included in the randomization. Group IIb children who were lost to follow-up or died prior to when the randomization was done will be included in the randomization, to avoid possible biases caused by their exclusion.

Group IIb children will be randomized with separate randomization strata for each site/institution. This will ensure that approximately the same proportion of controls are selected at each site/institution. Within each site/institution, the randomization will be blocked over time to ensure that at any point in the study, approximately the desired proportion of controls will have been selected at each site/institution.

The fraction of IIb patients/families selected to be in the control group (i.e., selection probability) is determined based on the expected number of Group IIb patients/families eligible to be randomized. Through April 30, 1993, a total of 466 Group II children had been enrolled in 432 families. It is estimated that 289 (67%) of the 432 families will have a Group IIb child eligible for randomization. Therefore, with the final enrollment of 600 Group II children, it is estimated that there will be 556 families, and an estimated 372 of these families will have a Group IIb child eligible to be randomized. Selection of 200 Group IIb controls from these 372 families results in an overall selection probability of 54%. An actual selection probability of 55% will be used.

4.2.2 Estimation of Incidence or Prevalence

To estimate the incidence or prevalence of specific pulmonary or cardiovascular complications in Groups I or II (or IIa and IIb), confidence intervals can be used. The precision of an estimated proportion, p, is given as the half-width of the confidence interval. For example, for a 95% confidence interval (CI) the half-width confidence interval (as a percentage) is equal to $100(1.96)\sqrt{(p(1-p)/n)}$, where n is the sample size. The maximum value of this width occurs when the proportion, p, is equal to 0.5. The half-width intervals for various sample sizes are given in Table 4.

Table 4

Sample Size and Precision of 95% Confidence Intervals for a Proportion of 0.5

Sample Size	1/2 Width 95% Interval
50	13.9%
100	9.8%
150	8.0%
200	6.9%
250	6.2%
300	5.7%
350	5.2%
400	4.9%
500	4.4%
600	4.0%

Therefore, Table 4 provides a guide to the maximum variability that would occur in estimating an incidence or prevalence rate. For example, if 25% of the 200 Group I children had PCP, then from Table 4 the precision (based on assumed 50% incidence) is 6.9%, whereas the actual precision is (based on a 25% incidence) 6.0%.

4.2.3 Comparison of Complication Rates

The sample size required to compare incidence rates of pulmonary or cardiac complications in two groups (Groups IIa and IIb, Hypotheses H1 and H2) can be estimated. The expected rates of various cardiac (Issenberg et al, 1985; Sherron et al, 1985; Lipshultz, Chanock et al, 1989) and pulmonary (Kattan, 1989 personal communication) complications in HIV-infected children (Group IIa) are given in Tables 5 and 6, respectively.

Table 5

Expected Cardiac Complication Rates in Children with HIV Positive Status

	CARDIAC		
Complication	Fetus	Neonate	2.5 Years
Left Ventricular Dysfunction	–	–	93%
Conduction Abnormalities	–	–	66%
Rhythm Disturbance	–	30%	70–77%
Pericardial Effusion	5–10%	10%	26%

Table 6

Expected Pulmonary Complication Rates in Children with HIV Positive Status

PULMONARY

Complication	All Ages
PCP	24%
Lymphoproliferative Lung Disease	16%
Bacterial Pneumonia	24%
At least one of the above	50%
MAI	6%
Tuberculosis	3%
Fungus	3%
RSV	3%

In children with HIV seronegative status, the incidence of each of these complications is very small, that is, less than 5% or even close to 0%. For the purpose of sample size estimation, we assume this rate is 5%. Rates in Tables 5 and 6 refer to incidence of at least one episode of the identified complication.

A two-sided test with 90% power will be assumed in calculating the sample size required for Group IIa and IIb for comparing complication rates in HIV-positive and negative children. A significance level of 1% is also assumed to help account for the multiple comparisons that will be made by examining many different cardiac and lung complications. Table 7 gives the sample size needed in each group to detect a difference between specific complication rates in HIV-positive children and HIV-negative children (with an assumed rate of 5%). Since the expected sample sizes in the HIV-positive and HIV-negative children are in the ratio of 120 to 480 at ages $\leq$ 6 months and 120 to 200 at ages > 6 months, sample size estimates were calculated using the method of Fleiss, Tytun and Ury (1980), which generalizes the equal group size method of Casagrande, Pike and Smith (1978).

Table 7

Sample Sizes for Comparing Specific
Complication Rates in HIV-Positive Children to a Rate of 5%
in HIV-Negative Children

Complication Rate in HIV-Positive Children	SAMPLE SIZE: On or before 6 mos of Age (4:1 Ratio of HIV Negative:Positive) HIV-Positive	On or before 6 mos of Age (4:1 Ratio of HIV Negative:Positive) HIV-Negative	After 6 mos of Age (1.67:1 Ratio of HIV Negative:Positive) HIV-Positive	After 6 mos of Age (1.67:1 Ratio of HIV Negative:Positive) HIV-Negative
10%	503	2012	673	1124
15%	159	636	220	367
20%	85	340	118	197
25%	55	220	78	130
30%	39	156	56	94
40%	23	92	34	57
50%	17	68	23	38
60%	12	48	17	28

With 120 HIV-positive children and 480 HIV-negative children followed from birth to 6 month of age, it will be possible to detect a difference between two complication rates as small as 17% (HIV-positive) versus 5% (HIV-negative). At follow-up times greater than 6 months of age, the number of controls is reduced to 200. With 120 HIV-positive children and 200 HIV-negative children, it will be possible to detect a difference between two complication ratios as small as 20% (HIV-positive) versus 5% (HIV-negative). If there is a 10% loss-to-follow-up rate each year among the HIV-positive and HIV-negative children, at the end of three years there would be approximately 84 HIV-positive and 140 HIV-negative children to compare. With these reduced sample sizes at three years of follow-up, it will be possible to detect a difference of 24% (HIV-positive) versus 5% (HIV-negative).

4.3 Recruitment and Follow-up

4.3.1 Recruitment Strategies

Recruitment of infants and children for this study will utilize the resources of the following ongoing pediatric AIDS research projects:

1. Pediatric AIDS Clinical Trial Group (ACTG), (NIAID),
2. Randomized Clinical Trial of Intravenous Immunoglobulin vs. Placebo in HIV Infected Symptomatic Children (IVIG), (NICHD), and
3. Women and Infants HIV Transmission Study (WITS), (NIAID and NICHD).

All five participating Clinical Centers have one or more of these NIH-funded ongoing research projects and have access to established patients and referral patterns for enrolling new patients. Moreover, all five Clinical Centers have built-in obstetrical components which will be recruiting high-risk pregnant women in large urban indigent health care facilities. Specific enticements will be used in this study to enhance recruitment and follow-up of patients; these include taxi-cab vouchers, day-care services and meal vouchers. In addition, Centers will dedicate ancillary personnel (nurses, social workers, aides) to this specific research; at least one member of the ancillary staff will be bilingual. It must be acknowledged, that the NIAID and NICHD treatment trials in HIV infected children, will have direct impact on the natural history of the cardiovascular and pulmonary complications of pediatric HIV infection, since most children will be taking some form of antimicrobial agent or antiretroviral agent. With the exception of the intravenous immunoglobulin study, it is unlikely that the newer protocols will contain a placebo arm. Therefore, careful medical histories and protocol enrollments must be recorded.

If not already available, at each of the five centers, a patient registry of HIV infected patients will be established, with appropriate safeguards for confidentiality such as locked files, key-word entry into computers and use of numbers rather than names. This list, designating numbers for patients, will be kept in each Clinical Center's Data Management office in a doubly locked desk/box. This study will contribute an additional source of patients: the HIV infected children of women recruited to the study. The physician on record for an eligible child will be contacted about the study and will be requested to ask the patient's parents or legal guardian (subject to local regulations) if they would like to be approached about the heart and lung program for HIV infected children. If they agree, a member of the study team will discuss the study protocol with the family and seek informed consent. At the entry point to the prenatal care system, HIV positive women will be informed by their obstetric care provider that there is a special program for HIV positive women and their offspring. (Each site will develop its own information sheet.) The women will be asked if they want to know more about it and, if they do, a member of the study team will describe the study protocol and seek informed consent.

At many sites there may be women who come in for labor and delivery with no prenatal care. Each Clinical Center's study coordinator will be in contact with the Obstetrics Service to identify such women. If the patients are HIV positive, they will be asked if they want to learn more about the program and if they consent, they will be approached by the study team for informed consent.

4.3.2 Enrollment

Neonates will be enrolled either prenatally or postnatally (up to 28 days of age for Group II). Every effort will be made to enroll infants prenatally so that maternal nutrition and health can be documented, fetal echocardiograms can be performed, and follow-up plans can be made. Given the state of indigent health care, however, it is likely that at least some children will have no opportunity for prenatal enrollment and these infants will be enrolled during the first two weeks of age. Patients enrolled will be entered into a Patient Registry that is to be approved by the Coordinating Center at the Cleveland Clinic.

In centers where this is feasible, women and their children will be seen at the same physical site. This site will be made as hospitable as possible, with play rooms, snacks, videos, etc. A coordinated team of physicians, clinical coordinators, social workers, nurse practitioners and clerical staff will be serving the study population, regardless of which or in how many studies they participate. Specialty evaluations and tests (e.g., echocardiograms, pulmonary functions, etc) will be done at that site as much as possible. For trips to radiology or other remote sites staff members will escort patients.

4.3.3 Management and Retention of Cohort

Once enrolled in the study, patient and parent(s) will be aggressively followed so that prospective studies can be accomplished. Although follow-up of any duration is difficult in an indigent population with chronic illness, poverty and drug abuse, special and dedicated social workers will maintain contact with families and assist them in keeping clinic appointments, obtaining social welfare benefits available and obtaining transportation to hospitals.

Study personnel will generate records of scheduled and missed appointments on a weekly basis. These listings will be used to make reminder calls, assure accurate scheduling and provide follow-up for missed appointments. Subject compliance with appointments will also be promoted by providing reimbursement for meals and transportation at each visit. Each site will review patient adherence data quarterly and report its findings to the collaborative group in order to identify and resolve problems on an ongoing basis.

4.4 Schedule of Routine Tests

Tables 8 thru 12 provide the schedule of routine tests, revision 6/10/93. In subsequent chapters the justification for each test is provided.

TABLE 8

Routine Test Schedule

GROUP I

	TIMING (MONTHS ON STUDY)																												
TEST	0	3	4	6	8	9	12	16	18	20	24	28	30	32	36	40	42	44	48	52	54	56	60	64	66	68	72	76	78
SERUM IG	x						x				x				x				x				x				x		
CD 3/4/8	x						x				x				x				x				x				x		
DHST(1)	x						x				x				x				x				x				x		
CMV CULTURE(2)																													
CMV SEROLOGY(2)																													
EBV CULTURE(2)																													
EBV SEROLOGY(2)																													
CBC	x						x				x				x				x				x				x		
ESR	x						x				x				x				x				x				x		
LDH	x						x				x				x				x				x				x		
SERUM STORAGE	x						x				x				x				x				x				x		
PULMONARY EXAM	x	x		x		x	x		x		x		x		x		x		x		x		x		x		x		x
SPO2	x	x		x		x	x		x		x		x		x		x		x		x		x		x		x		x
CXR	x						x				x				x				x				x				x		
PFT	x			x			x		x		x		x		x		x		x		x		x		x		x		x
CARDIAC ASSESS.	x		x		x		x	x		x	x	x		x	x	x		x	x	x		x	x	x		x	x	x	
EKG	x						x				x				x				x				x				x		
HOLTER	x						x				x				x				x				x				x		
ECHO	x		x		x		x	x		x	x	x		x	x	x		x	x	x		x	x	x		x	x	x	

(1) Children one year or older

(2) These tests are done only when clinically warranted

(Rev. 6/10/93)

57

TABLE 9

Routine Test Schedule

GROUP II (Months 0 - 6)

	TIMING FROM BIRTH (MONTHS)			
TEST	0	3	4	6
ELISA				
W. BLOT				
HIV CULTURE	x	x		x
SERUM IG	x			x
CD 3/4/8	x	x		
DHST				
CMV CULTURE(1)	x			x
CMV SEROLOGY(1)				x
EBV CULTURE	x			x
EBV SEROLOGY				x
CBC	x			x
ESR				x
LDH	x			x
SERUM STORAGE	x			x
PULMONARY EXAM	x	x		x
SPO2	x	x		x
CXR		x		
PFT				x
CARDIAC ASSESS.	x		x	
EKG	x			
HOLTER	x			
ECHO	x		x	

(1) CMV serology testing discontinued when child is positive for CMV infection. CMV culture continues to be taken and stored.

TABLE 10

Routine Test Schedule

GROUP IIa (MONTHS 8-78)

	TIMING FROM BIRTH (MONTHS)																										
TEST	8	9	12	15	16	18	20	21	24	28	30	32	36	40	42	44	48	52	54	56	60	64	66	68	72	76	78
HIV CULTURE																											
SERUM IG			x			x			x		x		x		x		x		x		x		x		x		x
CD 3/4/8		x		x				x			x		x		x		x		x		x		x		x		x
DHST			x						x				x				x				x				x		
CMV CULTURE(1)			x			x			x		x		x		x		x		x		x		x		x		x
CMV SEROLOGY(1)			x			x			x		x		x		x		x		x		x		x		x		x
EBV CULTURE			x			x			x		x		x		x		x		x		x		x		x		x
EBV SEROLOGY			x			x			x		x		x		x		x		x		x		x		x		x
CBC			x			x			x		x		x		x		x		x		x		x		x		x
ESR			x			x			x		x		x		x		x		x		x		x		x		x
LDH			x			x			x		x		x		x		x		x		x		x		x		x
SERUM STORAGE			x			x			x		x		x		x		x		x		x		x		x		x
PULMONARY EXAM		x	x			x			x		x		x		x		x		x		x		x		x		x
SPO2		x	x			x			x		x		x		x		x		x		x		x		x		x
CXR			x			x					x				x				x				x				x
PFT			x			x			x		x		x		x		x		x		x		x		x		x
CARDIAC ASSESS.	x		x		x		x		x	x		x	x	x		x	x	x		x	x	x		x	x	x	
EKG			x						x				x				x				x				x		
HOLTER			x						x				x				x				x				x		
ECHO	x		x		x		x		x	x		x	x	x		x	x	x		x	x	x		x	x	x	

(1) CMV serology testing discontinued when child is positive for CMV infection. CMV culture continues to be taken and stored.

TABLE 11A

Routine Test Schedule

GROUP IIb - CONTROLS (MONTHS 8-78)

	TIMING FROM BIRTH (MONTHS)																										
TEST	8	9	12	15	16	18	20	21	24	28	30	32	36	40	42	44	48	52	54	56	60	64	66	68	72	76	78
ELISA						x																					
W. BLOT						x[1]																					
HIV CULTURE																											
SERUM IG			x			x			x		x		x		x		x		x		x		x		x		x
CD 3/4/8		x		x				x			x		x		x		x		x		x		x		x		x
DHST			x						x				x				x				x				x		
CMV CULTURE(2)			x			x			x		x		x		x		x		x		x		x		x		x
CMV SEROLOGY(2)			x			x			x		x		x		x		x		x		x		x		x		x
EBV CULTURE			x			x			x		x		x		x		x		x		x		x		x		x
EBV SEROLOGY			x			x			x		x		x		x		x		x		x		x		x		x
CBC			x			x			x		x		x		x		x		x		x		x		x		x
ESR			x			x			x		x		x		x		x		x		x		x		x		x
LDH			x			x			x		x		x		x		x		x		x		x		x		x
SERUM STORAGE			x			x			x		x		x		x		x		x		x		x		x		x
PULMONARY EXAM		x	x			x			x		x		x		x		x		x		x		x		x		x
SPO2		x	x			x			x		x		x		x		x		x		x		x		x		x
CXR			x			x																					
PFT			x			x									x		x		x		x		x		x		x
CARDIAC ASSESS.			x			x			x		x		x		x		x		x		x		x		x		x
EKG			x						x				x				x				x				x		
HOLTER			x						x				x				x				x				x		
ECHO			x			x			x		x		x		x		x		x		x		x		x		x

(1) As required by Protocol

(2) CMV serology testing discontinued when child is positive for CMV infection. CMV culture continues to be taken and stored.

TABLE 11B

Routine Test Schedule

GROUP IIb - NON-CONTROLS (MONTHS 8-78)

	TIMING FROM BIRTH(MONTHS)					
TEST	8	9	12	15	16	18
ELISA						x
W. BLOT						x[1]

(1) As required by Protocol

TABLE 12

Routine Test Schedule

GROUP IIb MOTHERS

TEST	WITHIN ONE MONTH OF EDC
IMMUNOLOGIC STUDIES	x
CMV CULTURE	x
CMV SEROLOGY	x
EBV CULTURE	x
EBV SEROLOGY	x

Blank page

Blank page

5. Methods

5.1 Clinical and Laboratory Evaluation of Subject

Laboratory evaluation of subjects will be coordinated with other protocols at each institution to avoid duplication of studies, to minimize discomfort, and to decrease costs.

5.1.1 Initial Visit and Assessment of Mothers

Each of the five Clinical Centers is staffed by obstetrical departments which serve the health care facilities for indigent women. Through direct participation with the clinical centers, the obstetrical departments are already screening high-risk pregnant women for HIV-infection. High-risk mothers include those who are intravenous drug abusers and those with sexually transmitted diseases. A woman is considered to be at high risk for HIV infection if she admits to drug abuse, shows physical evidence of drug abuse, or has urine which tests positive for illicit drugs. Other factors that would place a woman into an HIV high risk group include: sexual relations with a person with HIV infection or with an intravenous drug abuser, country of origin of high prevalence rate of HIV infection, or multiple sexual partners. A woman is considered to have a sexually transmitted disease by her own admission or by testing of blood for typical infectious agents including hepatitis B.

It is estimated that 2 to 5% of all pregnant women delivering at indigent care facilities fall into the high-risk category. The collaborating obstetrical co-investigator(s) will inform the principal investigator(s) of the pending or recent delivery of a baby born to an HIV-positive mother, using the established paramedical system, (e.g., research nurse, data manager, social worker). A pediatric co-investigator or representative will visit the mother near the time of delivery to discuss enrollment of the child in this study.

For Group II, the initial visit of the mother-to-be or mother and infant will take place prepartum in the obstetrical clinic or postpartum in the hospital or pediatric clinic (up to 28 days after birth). If the mother has already been proved to be HIV-positive, documentation will be obtained and no further diagnostic tests to establish that fact need be done. Otherwise, blood specimens will be obtained for HIV antibodies, and/or HIV culture and/or special diagnostic tests (See Section 5.1.2). If the woman presents prepartum, the obstetrician will evaluate the pregnant woman for historical facts suggestive of HIV-infection and perform a physical examination to determine whether there are findings of primary HIV-infection (lymphadenopathy, hepatosplenomegaly, wasting) or opportunistic infection (oral candidiasis, pneumonitis, encephalopathy). If the woman is evaluated postpartum, the pediatrician will rely upon the woman's medical history and medical records for an evaluation of her HIV-status. The HIV-status will be defined postpartum by laboratory tests in the same manner as prepartum.

5.1.2 HIV Testing of Mother and Infants

The conventional tests for HIV antibody (ELISA and Western Blot) will be used to establish a diagnosis of HIV infection in pregnant women. If these tests yield indeterminant values other diagnostic tests will be employed (e.g., HIV culture). HIV cultures will be performed on Group II children at month 00 (not cord blood), 03 and 06. Two positive cultures will be necessary to assign a patient to Group IIa (infected). Two negative cultures, one of which must be the month 06 culture, will be necessary to assign a patient to Group IIb (noninfected). It is expected that approximately 20% of infants will identified as Group IIa.

ELISA will be performed on all Group IIb patients (control and non-control) at month 18 to confirm their HIV-negative status. A positive ELISA result will require performance of Western Blot for confirmation of HIV status.

5.1.3 Immunologic/Virologic Assessment of Infants in Group I

Infants in Group I will be those documented with vertical transmission of HIV-infection who are over 28 days of age. Approximately 200 infants and children total will be enrolled at the five centers. The time sequence of visits and immunologic/virologic tests are contained in Table 8. Although patients will be seen in clinic for other components of this study, the immunologic/virologic testing of Group I will be less intense and, in general, tests will be performed on an annual basis. The types of tests selected for Group I patients are those which will permit correlation of cardiac and pulmonary condition with HIV disease state as reflected in the degree of immunodeficiency and the extent of coinfection with CMV and EBV. Western Blot banding patterns have been associated with stages of HIV infection. For example, the loss of antibody to p24 Ag on Western Blots has been associated with advanced HIV disease (Falloon et al, 1989). Serum immunoglobulin (Ig's) are known to be a very sensitive index of HIV-infection in infants and children, frequently rising well beyond the normal ranges in early disease and falling in terminal disease (Falloon et al, 1989). Leukocyte markers (CD3, CD4, and CD8 cell counts) are perhaps the most sensitive in vitro assessment of the stage of HIV-infection, with CD4 cell counts of less than 200 indicative of a precarious state of health (Falloon et al, 1989). Delayed hypersensitivity skin tests (DHST) are a reliable means of testing specific T-cell recall of antigens, and can be used to determine anergy. Testing will be performed on children over 1 year of age. Cultures and serologic tests for CMV and EBV can be utilized in conjunction with tests of cardiac and lung function to determine the role these viruses play in accelerating the complications of HIV-infection. For Group I patients, these tests will be obtained when the patients are symptomatic, according to the schedule outlined in Table 8. Listed in Table 8 are clinically warranted tests (CBC, LDH, ESR, stored serum) which will be obtained on a 12 month schedule, except when intercurrent or chronic disease occurs. When patients develop intercurrent or chronic illness, these tests will be repeated (six times per year estimate); in addition, serum Ig's, lymphocyte subsets (CD3, CD4, CD8), DHST's-70°C serum (storage) will be repeated unless performed within 2 month's; CMV and EBV cultures will be obtained for chronic illness if previous cultures were negative. In addition, viral cultures for influenza, parainfluenza, RSV, and adenovirus

5.1.4 Immunologic/Virologic Assessment of Mothers and Infants in Group II

Infants in Group II (and their mothers) will be recruited into this prospective study on the basis of positive HIV ELISA and Western Blot tests. At least 80% of the HIV-positive mothers will be recruited prepartum to permit fetal echocardiography measurements to be made upon the fetus. Since the health of HIV-infected mothers may influence HIV transmission to their fetuses and thus predispose their living infants to lung and heart disease, several important immunologic and virologic tests will be performed on the mothers (Table 12). All of the mothers will have been previously tested for the presence of HIV by means of the ELISA and Western Blot tests. Mothers will be tested for CMV and EBV infection prepartum or at the time of delivery. Urine, will be cultured for CMV and throat washings (saliva) will be cultured for EBV. In addition, serologic tests for CMV and EBV will be obtained. The purpose of the virologic tests is not only to determine possible virologic cofactors in mothers but, more importantly to determine which infants are likely to acquire CMV and EBV by vertical transmission; to date vertical transmission of EBV has not been established. Infants receiving double, or triple, virus infection from their mothers are expected to display more severe and complex HIV disease, including cardiovascular and pulmonary complications (Falloon et al, 1989). For example, HIV-infected infants who develop LIP/PLH may have been congenitally infected with EBV as well as HIV. The state of maternal immunodeficiency secondary to HIV-infection may also play a crucial role in causing severe heart and lung complications of HIV-infection in developing infants. Thus, CD3, CD4, and CD8 cell counts will be assessed in mothers either before or near the time of delivery.

Table 9 displays the schedule of tests for immunologic function and viral infection for Group II patients from birth through 6 months. Twenty percent of these at risk infants are expected to be infected and identified by 6 months of age. The remaining 80% of the Group II patients are expected to lose seropositivity and be identified as Group IIb, thus forming a control cohort. Test schedules for Group IIa and IIb from months 8-78 are shown in Tables 10 and 11, respectively.

Group II infants will be cultured for CMV (urine) infection and EBV (throat washings/saliva) at 6 month intervals. Infants testing positive for CMV at birth or up to 28 days after delivery will be assumed to have acquired the infection on a congenital basis. After infants have become CMV positive (positive culture at any age or positive serology in children $\geq$ 12 months of age), urine will continue to be collected but will be stored at -70° (freezer) for subsequent culture analysis. CMV serology, however, will be discontinued. Urine specimens are to be frozen in antibiotic solution and 50% glycerine in vials as described

in Section 5.3.7.1.2 in the Manual of Operations. At an appropriate time in the future, these frozen urine specimens will be sent to a central laboratory (Dr. Gail Demmler) for CMV culture. Both EBV cultures and serologies will be continued after the child becomes EBV-positive.

The immunocompetence of the infants in Group II will be assessed on a prospective basis by measuring serum immunoglobulin levels, and leukocyte subsets (CD3, and CD4/CD8) and DHST. As discussed above, these selected tests of immunologic function convey information not only of the state of immunologic health of the infant but also are a measure of the extent of HIV disease in infected infants. Also listed in Table 9, 10 and 11 are four clinically indicated tests (CBC, ESR, LDH, and stored serum) which will be obtained on a 6 month schedule, except when intercurrent or chronic disease occur. When patients develop intercurrent or chronic illness, these tests will be repeated (six times per year estimate); in addition serum Ig's, lymphocyte subsets (CD3, CD4, CD8), DHST's, -70°C serum (storage) will be repeated unless performed within 2 months; CMV and EBV cultures will be obtained for chronic illness if previous cultures were negative. In addition, viral cultures for influenza, parainfluenza, RSV, and adenovirus will be obtained.

5.1.5 Diagnostic Evaluation of Symptoms not Associated with Pulmonary or Cardiovascular Complications

From time to time, infants infected with HIV will develop symptoms due to complications in organ systems other than the lung and heart. They may, for example, develop loss of motor milestones, develop frank encephalopathy, intractable diarrhea with malnutrition, or renal disease. These complications of HIV infection will be recorded in the medical histories of the children that will be obtained at 3 month clinic visits. Primary care physicians or other specialists will care for patients with these complications.

5.2 Pulmonary Evaluation of Subjects

In order to test the overall hypotheses and to accomplish the objectives proposed as they relate to the pulmonary system, the expected pulmonary complications will be followed:

a. Upper respiratory infection (rhinitis, pharyngitis, otitis)
b. Fungal pulmonary infection
c. Viral pulmonary infection
d. Bacterial pulmonary infection
e. Mycobacterial pulmonary infection
f. Pneumocystis carinii pneumonia
g. Lymphoproliferative lung disease
h. Other interstitial lung diseases
i. Chronic obstructive lung disease
j. Airway hyperreactivity
k. Upper airway obstruction
l. Pulmonary vascular disease

Criteria to diagnose these specific pulmonary complications are defined in Table 13.

We determined the frequency of specific tests by balancing the goal of early detection with cost and expected patient adherence. We recognized that frequent and invasive testing decreases patient adherence. The specific justification for each test and the rationale for the frequency of that test is addressed in the respective methods section and Table 14. Any studies already required and performed through other grants or contracts will be accessed and recorded to avoid duplication.

Table 13

Criteria for Diagnosis of Specific Pulmonary Complications

COMPLICATIONS	CRITERIA
a. Upper respiratory illness	Rhinitis, pharyngitis, or otitis diagnosed by appropriate history and physical examination.
b. Fungal pulmonary infection	Clinical evidence of pneumonia, new CXR abnormality, and tracheal secretion positive for fungal organism (by stain or culture).
c. Viral pneumonia	Clinical evidence of pneumonia, new CXR abnormality, and virus recovered from secretions, lavage fluid or lung biopsy without bacterial or PCP infection. Histologic evidence of invasive infection required for diagnosis of CMV.
d. Bacterial pneumonia	Clinical evidence of pneumonitis (i.e., cough, tachypnea, rales and purulent sputum) new chest x-ray abnormality, leukocytosis (>15,000 WBC predominant polymorphonuclear cells) with intracellular organisms from respiratory secretions or positive blood culture, or positive cold agglutinins.
e. Mycobacterial pulmonary infection	TUBERCULOSIS - New CXR abnormality, mycobacteria recovered from BAL or lung biopsy, or positive gastric aspirate. MAC - Detection of *Mycobacterium avium-intracellular* complex (MAC) by histologic assessment of biopsy or bronchoalveolar lavage fluid specimens; acid-fast organisms must be observed within recovered tissue alveolar (foamy) machrophages. Culture of these organisms from pleural fluid will also serve to define MAC infection of the lung.
f. Pneumocystis carinii pneumonia	Evidence of organisms from BAL fluid or open lung biopsy.
g. Lymphoproliferative interstitial lung disease	Evidence of interstitial lung disease by CXR and open lung biopsy histologically consistent for LIP or PLH (Joshi et al, 1985).
h. Other interstitial lung disease	Evidence of interstitial lung disease by CXR and pathology from open lung biopsy consistent with interstitial lung disease. BAL negative for PCP. (Edelson and Hyland, [1989]).
i. Chronic obstructive lung disease	Clinical findings, including expiratory airway obstruction on physical exam and/or chest roentgenographic evidence and evidence of hyperinflation in absence of clinical and/or laboratory finds of infection.
j. Airway hyperreactivity	Obstructive airway disease with clinical response to bronchodilators.
k. Upper airway obstruction	Abnormality by direct inspection and/or, appropriate clinical history.
l. Pulmonary vascular disease	Histologic evidence on open lung biopsy (Edelson and Hyland, 1989), or abnormality confirmed on cardiac catheterization or pulmonary angiography.
m. Bronchiolitis	Clinical findings consistent with bronchiolitis and absence of lobar

Table 14

Schedule of Pulmonary Studies

Month Pt. On Study (± 1 mo.)	Questionnaires			Physical exam including RR	CXR	Sp02 (a) with heart rate	PFT(b)	BAL	Bx
	Form 41(c)	Form 42	Form 43						
0 - 1	I	I		All	I	All	I		
3	II		All	All	II	All			
6			All	All		All	All		
9			All	All		All			
12			All	All	All	All	All		
18			All	All	II	All	All		
24			All	All	I	All	I, IIa		
30			All	All	IIa	All	I, IIa		
36			All	All	I	All	I, IIa		
42			All	All	IIa	All	All		
48			All	All	I	All	All		
54			All	All	IIa	All	All		
60			All	All	I	All	All		
66			All	All	IIa	All	All		
72			All	All	I	All	All		
78			All	All	IIa	All	All		
Intercurrent Illness				All	All			(e)	(e)
Chronic lung disease				(d)	(d)			(f)	(f)

NOTE: I = Group I IIa = Group IIa
II = Group II All = Group I and II

(a) ABG if SAT <96% or 96%-97% if clinically indicted.
(b) Refer to Section 5.2.4.4. for details of PFT testing for specific age groups.
(c) In addition, complete with any change in household.
(d) As indicated.
(e) Refer to Intercurrent Illness Algorithm (Figure 1).
(f) Refer to Chronic Lung Disease Algorithm (Figure 2).

5.2.1 Perinatal

The maternal history, prenatal and birth history, neonatal complications, and interventions are cofactors as listed in secondary hypotheses four and five. The perinatal form will be completed once on every patient and allow systematic collection of this information. We recognized that information will be obtained easily from Group II patients. Group I patients, especially those in foster care, may have missing data which will be handled appropriately during statistical evaluation of this form.

5.2.2 Scheduled Pulmonary Evaluation

The schedule of routine pulmonary evaluation is constructed to gather baseline data from patients prior to illness and to monitor the patients at an interval expected to allow early detection of pulmonary complications. The median age of diagnosis of HIV is 13 months, with the median age of onset of lymphoproliferative lung disease at 17 months. A relatively frequent interval of monitoring (every 3 months), will allow early detection of these pulmonary complications. Less invasive studies constitute this screening battery. Clinical illness prompts initiation of the algorithms for evaluation of an intercurrent illness or chronic lung disease. Questionnaires (Qst) were developed to follow the history of pulmonary symptoms, clinical course, and clinical care for Group I and Group II. The perinatal form is discussed in Section 5.2.1. The environmental Qst is completed at entry into the study and with each change of household during the study for both groups. The basic respiratory Qst is completed at entry into the study for Group I patients. The interval respiratory Qst is completed each visit following enrollment. During each patients first year, the patient is seen every 3 months; during the remainder of this study, the patient is seen every 6 months. A physical examination form will be completed at all of these visits. The respiratory rate is always recorded during sleep or quiet breathing. Oxygen saturation (SpO_2) will be performed at every visit as outlined in Section 5.2.4.4. Pulmonary function testing will be performed biannually on Group I and II patients. Details of pulmonary function testing for specific age groups are found in section 5.2.4.4. A DTPA clearance scan will be performed on all patients at six-month intervals. Scans will be discontinued after 18 months in HIV negative patients (Group IIb). (DTPA studies were discontinued on February 10, 1993 on the recommendation of the Steering Committee).

5.2.3 Diagnostic Evaluation of Symptoms or Findings Suggestive of Lung Disease

All diagnostic evaluations will be performed in the Clinical Center. Procedures will follow the methods devised for purposes of the protocol (Section 5.2.4). The diagnostic evaluations may be triggered by the occurrence of symptoms, signs, abnormal laboratory tests, or chest x-ray (noted either at the time of a scheduled evaluation or between evaluations).

5.2.3.1 Intercurrent Illness

The patients being studied are at a high risk of developing significant

pulmonary problems. Therefore, any one of the following symptoms, signs and test results will trigger a respiratory evaluation:

1. Unexplained cough for more than 5 days
2. Oral temperature > 38° C for more than 5 days or rectal temperature > 38.5° C for more than 5 days
3. Tachypnea (See Section 5.2.4.1)
4. Oxygen saturation < 96%
5. Abnormal chest x-ray
6. Crackles on auscultation
7. Appearance of digital clubbing

As part of the orientation and at each routine clinic visit, the caretaker of the subject will be alerted to the symptoms and instructed to contact the Clinical Center should they develop. If the patient is febrile with localizing extrapulmonary symptoms and no pulmonary symptoms, no further respiratory evaluation will take place. The patient will be contacted by telephone or visit after 48 hours to ascertain if the symptoms have resolved. If the patient remains symptomatic, he/she will be evaluated.

The respiratory symptom evaluation visit will include:

1. Interval visit questionnaire and physical examination
2. Chest radiograph (PA and lateral views)
3. Arterial blood gas
4. Blood culture
5. Respiratory cultures:
   - Adenovirus
   - RSV
   - Parainfluenza
   - Influenza (during the epidemic season)
6. Laboratory studies:
   - CBC, ESR, LDH, at each illness;
   - CD4/CD8, Serum IgG, serum frozen at −70° C (if none in previous two months)
7. Skin tests (if none in previous two months):
   - PPD
   - DHST
8. Cardiac physical examination, as required (see Section 5.3.8)

The intercurrent illness algorithm will be followed after the respiratory symptom evaluation visit (Figure 1). The algorithm is intended to maximize the possibility of making a diagnosis while minimizing invasive studies which might decrease patient adherence. If the symptom evaluation visit reveals a new abnormality on chest x-ray or tachypnea, further evaluation will be dependent upon presence or absence of hypoxemia. If a blood culture is positive with a new radiographic abnormality, the patient will be treated for a bacterial pneumonia with antibiotics. If a clinical response is not observed within 72 hours or if TMP-SMX is started, a bronchial lavage will be performed to rule out PCP. If the

patient remains symptomatic despite two weeks of appropriate antibiotic therapy, an open lung biopsy will be performed. We will wait up to five days after instituting empiric antibiotic therapy prior to performing a bronchial lavage oran open lung biopsy on patients with focal consolidation who are hypoxemic. An open lung biopsy will be performed within 24 hours if the radiograph shows diffuse interstitial lung disease with a negative BAL and the patient is hypoxemic.

Bronchoscopy/BAL

Patients will have bronchoscopy and bronchoalveolar lavage (BAL) according to the algorithm (Figure 1). BAL will be performed on hypoxemic patients or those unresponsive to appropriate treatment for up to 2 weeks or experiencing worsening symptoms. The fluid will be handled as per Section 5.2.4.6. The lavage should preferably be done within 48 hours of the documentation of hypoxemia or within 48 hours of starting TMP-SMX.

Open Lung Biopsy

An open lung biopsy will be done if a bronchial lavage is non-diagnostic or if treatment has failed despite a diagnostic bronchial lavage. The specimen will be processed as outlined in Section 5.2.4.7. All patients who undergo open lung biopsy for any reason will have a BAL the same day so that direct comparison of the value of these methods may be made and recommendations for their use in the future may be clarified.

Follow-up

Patients with intercurrent illness or chronic lung disease will continue to receive the scheduled follow-up after the illness has resolved or stabilized. In patients who are hospitalized, a discharge evaluation will be made. This will include:

a) Physical examination
b) Chest radiography
c) Oxygen saturation and/or arterial blood gas

5.2.3.2 Chronic Lung Disease (CLD)

Patients with CLD will first be identified with an intercurrent respiratory illness and will have had the evaluation outlined previously (intercurrent illness algorithm). The chronic lung disease algorithm will be followed for patients with an abnormal chest x-ray or tachypnea persisting for more than 2 weeks (Figure 2).

Cultures for CMV and EBV will be done if previously negative. Bronchoscopy and BAL will be performed on hypoxemic patients and normoxemic patients with increased tachypnea or deterioration in the chest radiographs over the 2 week period of observation. In those patients whose radiograph remains unchanged, the diagnosis of interstitial lung disease (LIP/PLH) is the most likely (Rubenstein, 1986). These patients will first have a BAL and, if negative, an open lung biopsy.

The CT scan should be performed in children with persistent infiltrate for six month duration, specifically persistent consolidation in one area of the lung. A CT scan can clearly delineate areas of bronchiectasis in these children and is encouraged in cases of persistent pneunonia when no specific diagnoses has been made.

Figure 1
Intercurrent Ilness Algorithm

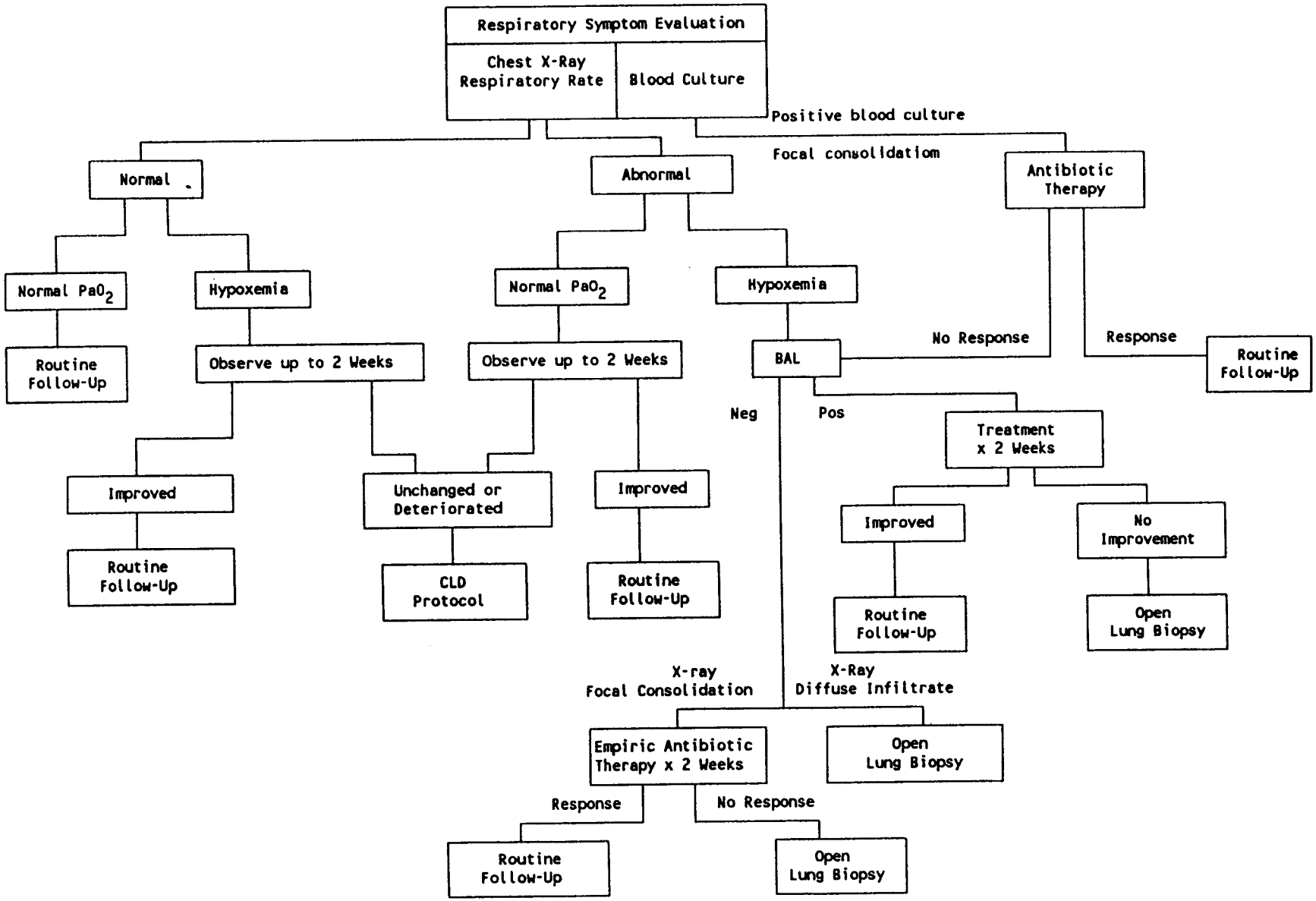
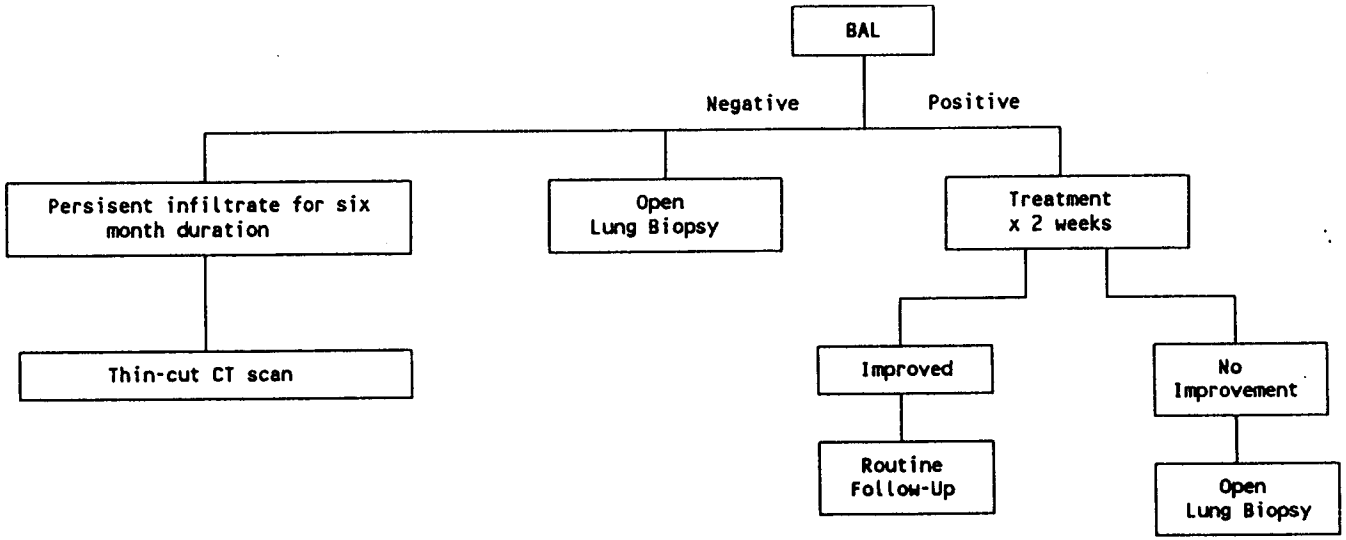


Figure 2
Chronic Lung Disease Algorithm

If the chest x-ray is unchanged or worse, or there is tachypnea or hypoxemia persisting for more than 2 weeks, the CLD algorithm will be followed. If the patient has a persistent infiltrate for six month duration, a thin-cut CT scan should be performed to diagnose bronchiectasis.



5.2.4 Pulmonary Assessment Methods

5.2.4.1 Respiratory Rate

Rationale:

The rationale for these measurements of respiratory rate is in part time honored, following the traditional clinical belief that respiratory rate parallels the course of lung disease and that the observations in infants that abnormalities of gas exchange are paralleled by increases in respiratory rate (Reynolds, 1963; Simpson, 1974).

Method:

Respiratory rate will be measured at each laboratory and clinical visit while the child is asleep or quietly breathing while awake. Observations will be carried out for a full minute by a trained observer. Based on data summarized in the literature (Gaultier, 1985), mean frequencies and the upper limit of normal (estimated to be 3 standard deviations) are given in Table 15.

Table 15
Respiratory Rates

Age (months)	Mean (breaths/min.)	Upper Limit (breaths/min.)	Coef. Variation (% mean)
0 - 2	45	80	25
3 - 4	36	60	20
5 - 6	31	50	20
6 - 12	25	36	15
13 - 24	24	35	15

5.2.4.2 Chest X-rays

Rationale:

Chest x-rays obtained at intervals provide an opportunity to detect pulmonary infiltrates at an early stage and to record the radiologic response to treatment or the progression of the pulmonary disease in the absence of a response to treatment.

Frequency of Study:

Chest x-rays will be routinely performed at enrollment and months 12, 24, 36, 48, 60 and 72 on Group I patients; months 3, 12, 18, 30, 42, 54, 66 and 78 on Group IIa patients; and months 3, 12, 18 for Group IIb. Chest x-rays will also be performed when clinically indicated as part of diagnosis and treatment of intercurrent respiratory tract illnesses; the latter will be charged to the study. (Prior to 6/10/93, CXR test schedule for Group I and Group IIa was month 3, 9, 18, 30 and 42; Group IIB was month 3, 9 and 18).

Method:

Inspiratory chest x-rays will be obtained in frontal and lateral planes, using routine techniques as performed at the individual centers.

5.2.4.3 Determination of Oxygenation and Pulmonary Gas Exchange by Pulse Oximetry and Arterial Blood Gas Analysis

Rationale:

Arterial blood gas analysis is the most sensitive laboratory method for detecting abnormalities in pulmonary gas exchange and for monitoring gas exchange adequacy over time. However, this is an invasive laboratory test. Recently, it has been possible to estimate the adequacy of oxygenation in small infants by pulse oximetry (SpO_2), a non-invasive, continuous estimate of hemoglobin oxygen saturation (Fanconi, 1988; Praud et al, 1989). We propose to monitor oxygenation with this test and to reserve arterial blood gas analysis for instances in which pulse oximetry is either indeterminate (see interpretation), inaccurate (e.g., methemaglobinemia, skin color) or clearly below the normal range.

Frequency of Study:

Pulse oximetry will be performed at each study interval, i.e. every three months during the first year and twice yearly for the remainder of the tenure of the project. Arterial blood gas analysis will be performed when SpO_2 is abnormal in order to determine the extent of the defect in oxygenation and to define whether ventilation, i.e. carbon dioxide elimination, is also impaired. During clinic visits for evaluation of intercurrent illness, arterial blood gas analysis, rather than SpO_2 will be performed.

Method:

Pulse oximetry is based on a modification of the Beer-Lambert law. In this test, the absorbance of a light of known wavelength transmitted through the arterial blood is measured. In clinical pulse oximetry, the arterial pulsatile component is measured through analysis of absorbance in the red and infrared wavelengths and, thus, theoretically allows determination of oxygen saturation of arterial blood. With the eight wavelength band pulse oximeters, the correlation has been excellent (Strohl et al, 1986; Praud et al, 1989). Recent clinical monitors utilize the measurement of transmission at two wavelengths.

Use of multiple wavelength analysis avoids the confounding variables of capillary and venous blood and eliminates the necessity of arterializing blood flow through a limb by warming it. However, the problems encountered by the inability to exclude the influence of carboxyhemoglobin and methemoglobin are not overcome with monitors utilizing measurement of transmission at only two wavelengths (Tremper, 1989; Praud et al, 1989). Methemoglobin concentrations greater than 30% will overwhelm the hemoxyglobin signal and cause the oximeter to read 85% regardless of the true saturation with oxygen.

In practice, however, the two major causes of artifact in the pulse oximetry method are motion and extraneous light (Nellcor Incorporated, 1986; Yelderman and New, 1983). The light detector must be in continuous contact with the skin of the patient and must be totally shielded from extraneous light (e.g. room or examination lighting) since as the detector emits current whenever light excites its surface. There is a correction logic wired into the circuit as the oximeter first turns on red light emitting diode (LED) then infrared light emitting diode, and finally both red and infrared to filter out the room light. This cycle is repeated continuously, hundreds of times a second. Light and especially motion can still cause troublesome artifacts. While in stable infants and children pulse oximetry has been a reliable measurement of oxygen saturation, in patients who are critically ill and/or experiencing peripheral circulatory problems, the absence of adequate peripheral blood flow can also lead to inconclusive assessment of oxygen saturation (Swedlow and Stem, 1983; Ramanathan et al, 1987; Taylor and Whitman, 1986; Fanconi, 1988). In an attempt to control for this artifact, impedance heart rate monitoring will be performed concurrently, but independently of pulse oximetry and the SpO_2 will only be accepted when the oximeter heart rate is within three (3) beats/minute of the impedance heart rate. The oximeter to be used in this study yields oxygen saturation measurements which, when compared to co-oximeter measurements of oxygen saturation, overestimates saturation when the arterial oxygen saturation is less than 96% (Fanconi, 1988; Praud et al, 1989).

Equipment:

1. Nellcor pulse oximeter (Nellcor Incorporated, Hayward, California)-model N-10 battery-operated, with display of pulse, oxygen saturation and heat sensitive paper recording of a ten minute study of pulse and saturation.

2. Impedance cardiopulmonary monitor

Interpretation:

The values of SpO_2 fall into three ranges:

1.	Normal	-	SpO_2 >97%
2.	Indeterminate	-	SpO_2 96 - 97%
3.	Abnormal	-	SpO_2 < or = 95%

For the SpO_2 study to be considered valid, the pulse corresponding to a SpO_2 value must be accurate. Therefore, for each minute, during the 10 minute observation period, the pulse and the corresponding SpO_2 must be recorded. When review of the 10 minute record reveals the pulse in any minute to vary by more than 10% of the average pulse for the observation period, the SpO_2 for that minute is likely to be inaccurate. Another method to confirm the accuracy of the

pulse determined by the oximeter is to perform simultaneous impedance monitoring of the heart rate. This method is the most accurate method for measuring heart rate. The pulse value from the oximeter must be within 2% of the impedance value or the oxygen saturation value is invalid.

When the oximetry recording is considered accurate and free from pulse artifact described above, and a SpO_2 value is indeterminate for 3 of the 10 minute monitoring period, another 10 minute observation period is indicated. When there is an abnormal tracing or a second 10 minute observation period yields indeterminate values for 3 of the 10 minutes, the patient should receive an arterial blood gas study. An exception to this guideline may be indicated in some patients closely followed by the physician, when the patient has a normal physical examination, a normal sleeping respiratory rate and a normal chest roentgenogram.

Blood gases will be drawn with patients breathing room air unless they require continuous oxygen administration. The oxygen flow at which the blood gas is drawn will be noted and reported.

Normal blood gas values on room air include:

pH	–	7.37 – 7.42
$PaCO_2$	–	37 – 42
PaO_2	–	>80 torr or an associated $A\text{-}aDO_2$ gradient of less than 15 (after 1 month of age; normal PaO_2 at birth and during the first month after birth = or > 70)

5.2.4.4 Pulmonary Function Studies

The infant pulmonary function studies to be done from infancy until 5 years of age consist of measurements of total respiratory system resistance and compliance, measurements of forced expiratory flow from partial expiratory flow volume (PEFV) curves and measurements of functional residual capacity (FRC). All clinical centers will use equipment produced by the same manufacturer (SensorMedics 2600). From 3–5 years of age, mini Wright peak flow studies will be attempted.

Spirometry testing will be done on children > 61 months of age using a mini-Wright peak flow meter and a Wedge spirometer, pneumotachograph or rolling seal spirometer. Measurements will include FVC, FEV_1, $FEF_{25-75\%}$ and FEF.

Rationale:

The major rationale for selection of pulmonary function testing with SensorMedics 2600 equipment include: they can be performed in infants; they have been shown to be abnormal in pediatric diseases affecting airways and/or lung parenchyma (Tepper 1984; Tepper 1986, Tepper 1987; Hiatt, 1988; Godfrey, 1983); they are likely to be accomplished within the time framework of a single sedation with chloral hydrate; and the studies, as outlined, are less invasive than dynamic or static lung compliance which require placement of an esophageal catheter (Heaf 1986).

Limitations:

The major limitations of pulmonary function testing in children 2–3 years of age are two: Not all tests may be accomplished at each testing because of

sedation failures; and growth causes substantial and sometimes overriding changes in values which may mask slow rates of decline of lung function. It must be remembered that infants essentially triple their lung volumes, halve their respiratory system resistance and triple their respiratory system compliance in the first year of line (Colin et al., 1989, Master, 1987).

Frequency of Tests:

The frequency of testing has been selected to insure points at appropriate growth intervals; yet, we have been mindful of what is practical. Testing will be performed at six-month intervals. The equipment to be used (SensorMedics 2600, Mini-Wright Peak Flow/Meter, Wedge spirometer, pneumotachograph or rolling seal spirometer) varies according to the age of the child (see schedule in Manual of Operations, page 59).

Methods:

PEFV Curves (Shaeffer and Cerny, 1985; Adler and Wohl et al, 1978; Godfrey et al, 1983; LeSouef, 1986; Taussig et al, 1982; Tepper et al, 1987)

Following sedation with 80-100mg/kg of chloral hydrate administered in a small amount of formula, the infant is studied supine in an appropriately fitting inflatable jacket (Hammersmith Hospital). Pressure is measured in the jacket. Flow is measured with a pneumotachograph attached to an anesthesia face mask placed over the infant's mouth and nose. To obtain PEFV curves, the jacket is pressurized to augment exhalation. At least 60 seconds is allowed between three successive PEFV maneuvers to limit the effect of volume history on flow (Morgan et al, 1989).

The following signals are recorded:

1. Jacket pressure
2. Flow
3. Volume

Peak flow at FRC, the slope of the flow-volume relationship over the mid-tidal range, and mid-tidal volume flow are calculated from a minimum of three curves which are superimposable. The data collection, calculation of results, and calibration of equipment will be carried out by techniques described in the Manual of Operations.

Measurements of compliance and resistance of the respiratory system (technique of Mortola et al, 1982; with modifications LeSouef, 1984 A&B).

This test is performed on supine, sedated, sleeping infants. An anesthesia mask is applied to the infant's face and connected to a pneumotachograph permitting measurement of respiration during quiet breathing. At end inspiration, the airway is only occluded for about 200 milliseconds and then the infant exhales passively. The assumption is that occlusion produces relaxation
and that the system relaxes to a passively determined volume (Zin et al, 1982). Ten to 15 occlusions are performed, and compliance is calculated. Each subject's reported result is the average of at least 3 acceptable tracings.

Determination of Functional Residual Capacity (FRC) by the Nitrogen Washout Method (Gerhardt, 1985; Ronchetti, 1975)

FRC is measured by a variation of the open circuit adult technique. The

volume of nitrogen washed out of the lung is obtained as the integral of nitrogen concentration measured in a constant flow washout circuit. Initial alveolar nitrogen concentration is calculated based on the assumption that the fraction of N_2 is .79 while breathing room air. The apparatus consists of a low dead space mask, a constant flow source of 100% O_2, a patient switch-in valve, a mixing chamber and a nitrogen analyzer. The constant flow rate is adjusted to slightly exceed the infant's peak inspiratory flow rate. In general washouts will last two minutes but can be prolonged.

5.2.4.5 Aerosolized Tc-99m DTPA Scintigraphy (DTPA studies were discontinued on February 10, 1993 on the recommendation of the Steering Committee)

Rationale:

This study may offer the opportunity of detecting early lung injury non-invasively. Tc-99m DTPA is a small molecular weight solute of approximately 500 daltons and when deposited in the alveolus, initially adheres to the pulmonary epithelial surface. The radiotracer then diffuses across this membrane into the interstitial space and then into the capillary to be excreted by the kidney. In this way, the only significant barrier to absorption of this radiotracer is the pulmonary epithelial membrane. Thus, changes in the rate of absorption of Tc-99m DTPA have been shown to correlate with damage to the pulmonary epithelial membrane; the more damaged the membrane, the faster the clearance of radiotracer from the lung. This method of assessing the integrity of the pulmonary epithelial membrane has been used in numerous animal models of pulmonary injury and disease states affecting adults and children (Coates, 1986; Meignan et al, 1988). More recently, preliminary studies have suggested that this technique may be the investigation of choice in the non-invasive detection of Pneumocystis carinii infection in HIV positive patients (O'Doherty, 1987). Although the results are affected by changes in lung volume, we will, in part, control for this by measuring functional residual capacity in the same position at a time relatively close to that of this study by using the nitrogen washout technique.

Method:

The patient will be imaged while lying in the supine position over the face of the gamma camera. Tc-99m DTPA is rebulized using a conventional mask until a count of approximately 50,000 is obtained. The washout is then recorded dynamically with continuous 15 seconds computer frames; the more rapid the disappearance or clearance, the more permeable the epithelial layer. Regions of interest for both whole lung and the peripheral third of each lung are drawn and time activity curves are generated for each lung. A half clearance time for each lung is derived.

Radiation Dosimetry

The radiation exposure from aerosolized Tc-99m DTPA scintigraphy is quite low. In adults in whom a typical dose of 750 microcuries reaches the lungs, the pulmonary radiation dose is 50 to 75 mrad per study (5×10^{-4} to 7.5×10^{-4} Gy) (Alderson et al, 1984). Total radioactivity delivery to the lungs of infants will be less, probably by more than a factor of two (Alderson et al, 1974).

Frequency of Testing:

These studies will be performed at six-month intervals. For Group I patients the studies will begin at enrollment. DTPA studies for Group II will begin at month six, but will be discontinued after 18 months for patients found to be in the Group IIb cohort. (Refer to Table 14.)

5.2.4.6 Bronchoalveolar Lavage

Rationale:

Bronchoalveolar lavage fluid has proved to be an invaluable tool for the identification of pulmonary complications associated with HIV infection. Patients in both groups will undergo flexible bronchoscopy and/or catheter placement with bronchoalveolar lavage only when clinically indicated. Specifically, clinical symptoms and signs of respiratory distress that occur in conjunction with abnormal gas exchange, or an abnormal radiograph unresponsive to management as outlined in the standard study protocol or in the intercurrent illness and chronic lung disease protocol, will indicate the need to obtain BAL fluid.

Methods:

Following informed consent, sedated patients will be examined under local anesthesia. Oxygen saturation will be maintained with supplemental oxygen as necessary. Bronchoscopy will be performed to evaluate the upper airway, tracheal anatomy, and airway dynamics systematically. Lavage will be performed following wedging of the bronchoscope into a segmental bronchus of the most involved lobe documented by radiograph or the right lower lobe bronchus if the radiograph is diffusely abnormal. Lavage will be performed using aliquots or sterile room temperature non-bacteriostatic normal saline (1-2 ml/kg or a maximum of 10 ml aliquots).

Intubated patients will be examined following the above guidelines. Ventilation will continue through a Bodai side arm and the bronchoscope will be inserted through the endotracheal tube to enter the distal airways directly.

Intubated patients whose endotracheal tube is too small to allow use of the flexible bronchoscope (< or = 4.0) will have an appropriately sized catheter inserted through the endotracheal tube and wedged. Aliquots of sterile non-preserved saline will be instilled and suctioned in a manner identical to that using the bronchoscope.

Specimen Analysis:

Bronchoalveolar lavage specimens will be treated according to established institutional guidelines for the handling of infectious material. The lavage fluid will be sent to the laboratory where it will be sampled for cell counts and differential cell count, cultured, and examined with a variety of stains to evaluate the cells and the presence of organisms. Any cells and fluid which are not used in these diagnostic procedures will be appropriately treated and stored so that it can be used for ancillary studies at a later time.

5.2.4.7 Lung Biopsy Processing and Analysis

Rationale:

Lung biopsies will be done, whenever appropriate, (See Figures 1 and 2) to establish the diagnosis of the disease processes involving the lung. Infectious complications, although seen less in children than in the adult, are a frequent cause of morbidity and mortality. The biopsy findings, in addition to providing diagnostic information in each case, will provide information to the study concerning the relative frequency of infectious and non-infectious pulmonary complications. In addition to their diagnostic utility, systematic examination of lung biopsy tissue for the presence and location of such agents as EBV and CMV will help establish their role in the pulmonary lymphoproliferative lesions, seen almost exclusively in pediatric patients.

All patients who undergo open lung biopsy for any reason will have a BAL the same day so that direct comparison of the value of these methods may be made and recommendations for their use in the future may be clarified. (see Section 5.2.3.1)

Methods:

Open lung biopsy will yield a specimen approximately 1 X 1 X 1 cm. The biopsy should be obtained from an area of active disease as judged radiographically and in general should not be obtained from the tip of the lingula or the tip of the right middle lobe. There is considerable evidence in adults, less in children, that the changes in these areas are disproportionate and may lead to a faulty impression of the severity of the pathologic processes.

The biopsy will be divided for culture, light microscopy, electron microscopy and a portion will be frozen. The light microscopic sections will be examined with routine hematoxylin and eosin stains and with stain to identify organisms and structural elements. All biopsies will be processed such that portions will be suitably preserved for ancillary studies including examination for EBV, CMV, HIV, etc. by in situ and immunohistochemical methodology.

5.3 Cardiovascular Evaluation of Subjects

5.3.1 Fetal Echocardiography (Fetal Echocardiography was discontinued on December 19, 1992 on the recommendation of the Policy, Data and Safety Monitoring Board.)

Hypothesis and Rationale for Fetal Echocardiography in Mothers with HIV Infection

We will determine whether the incidence of cardiac complications is elevated in the population of infants in which there is vertical transmission of HIV infection from mother to fetus. In view of the high incidence of HIV infection in the newborn offspring of mothers with the infection (Ryder et al, 1989, Blanche et al, 1989) and the observation that postnatal echocardiograms may be abnormal even when there are minimal systemic manifestations of HIV infection postnatally (P1 grouping) (Lipshultz, Chanock et al, 1989), it is possible that with fetal echocardiography changes in cardiac function may be detectable. Identification of HIV-specific in utero changes in cardiovascular function may be confounded by the effects of other vasoactive substances, including cocaine. Schmidt et al (1989) have recently described echocardiographic methods which have defined abnormal cardiac function in fetuses who had cardiomyopathy detected in the second and third trimesters of pregnancy. The potential to define fetal cardiac functional abnormalities in HIV positive fetuses is therefore possible.

Study Periods:

28-38 menstrual weeks of gestation

Ultrasound Equipment Requirements:

High resolution ultrasound scanners employing 3.5 and 5.0 mHz frequency transducers with capabilities for pulsed Doppler ultrasound are required. Pulsed Doppler requirements include ability for baseline shift and full spectral output. On-line or off-line analysis of the Doppler tracing are required to fulfill protocol measurements.

All studies are recorded on 1/2" standard (or super) VHS recording transcribed and to standard 1/2" VHS videotape for record analysis and quality control. Tape studies are numbered with examining center's initials, the preassigned fetal code number, and clear reference to starting and ending times recorded.

Measurements:

Fetal age and growth - Menstrual age is reported for comparison with the following measurements to determine fetal age and growth:

1. Femur length (Ott, 1984)
2. Skull biparietal diameter (Jentry, 1984)
   (These are used to assess gestational age from standard obstetrical ultrasound tables

Umbilical Artery Doppler:

An index of umbilical arterial pulsatility, such as peak systolic to diastolic ratio (the A/B ratio), is determined from the umbilical vessels by placing the sample volume as close to the placental insertion as possible (Abramowicz et al, 1989; Campbell et al, 1987; Schulman et al, 1984; Stuart et al, 1980; Trudinger et al, 1985; Trudinger, Giles, and Cook, 1985; Trudinger, 1987). This measurement has been used as an indication of fetal dysmaturity.

Fetal Cardiac Measurements

1. Ventricular wall thickness and cavity dimensions:

   From a 4 chamber view, free walls of the right and left ventricular and septal thicknesses are measured from an end diastolic frame (maximum dimension) at the level just below the annulus of the atrioventricular valves (Shime et al, 1986; Schmidt et al, 1989; Allan et al, 1985; De Vore et al, 1984). Left and right ventricular cavity dimensions are also made at this time. The end systolic cavity dimensions (minimum dimension) is measured from the same points as end diastolic dimensions. From these cavity dimensions fractional shortening index (FS) of both the left and right ventricle is calculated from the formula % FS = (EDD-ESD)/EDD where % FS is the percentage of shortening of the ventricle and EDD and ESD are the end diastolic and end systolic dimensions of the respective ventricles (Schmidt et al, 1989). Cavity dimensions and wall thicknesses are compared to established normal standards (Allan et al, 1985; De Vore et al, 1984; Shime et al, 1986).

If it is not possible to obtain 4 chamber view of sufficient quality, dimensions is obtained from a short axis view at the level of the mitral valve leaflets near their annular attachments. Ventricular and septal end diastolic and end systolic wall thickness are measured from this view. Again, using maximum dimensions obtained to represent the end diastolic frame, slight cranial angulation is required to measure the tricuspid valve annulus diameter. Right ventricular wall dimension is measured just distal to the annulus in this plane. End diastolic and end systolic cavity dimensions of the right ventricular cavity are also measured at this position.

2. Aortic and pulmonary artery dimensions:

Aortic and pulmonary diameters will be measured in systole from several planes including 4 chamber planes with cranial tilt, short axis views and sagittal views of the great vessels. The systolic portion of the cardiac cycle will be used to make these measurements.

3. Cardiac Doppler Measurements:

Doppler spectral/velocity data will be recorded with the Doppler reference cursor parallel to axial flow or with on-line angle correction of discrepancy between Doppler reference cursor orientation and axial flow across the 4 cardiac valves (De Smedt et al, 1987; Meijboom et al, 1985). For the atrioventricular valves, peak early diastolic velocity (E) and peak late atrial velocity (A) will be measured, the E:A ratio, and diastolic velocity time integral determined (Reed and Meijboom, 1986; Reed, Sahn et al, 1986; De Smedt et al, 1987; Meijboom et al, 1985). For all valves, the velocity time integral (VTI) and peak velocity will be measured averaging 3 cycles. Heart rate will be calculated from the aortic signal. Combined left and right ventricular outputs may then be calculated from the formula $Q=(\pi/4)(D)^2 \times VTI \times HR$ (Goldberg et al, 1985).

In addition, the flow signal velocity area proximal to the atrioventricular valves is interrogated to determine the presence of mitral and tricuspid regurgitation. Severity is assessed by qualitative grading of the regurgitant jet. The presence or absence of pericardial effusion is noted. Assessment for hydrops fetalis is made (Chin, 1988).

The proposed number of fetal echocardiographic studies is shown in Table 16.

Table 16

Proposed Patient Number of Fetal
Echocardiographic Studies for All Centers Combined

	Year 2	Year 3	Year 4	Year 5	Year 6
Patients acquired Group II	252	252	0	0	0
Maximum number of fetal echocardiograms Group II	428	570	214	0	0
Expected number of fetal echocardiograms* Group II	321	428	160	0	0

*This assumes that 1.5 studies on the average will be acquired per patient due to differences in the gestational age at presentation.

5.3.2 Postnatal Cardiac Protocols

Hypotheses and Rationale for Cardiac Evaluation in the Neonate, Infant, and Child

In order to test the overall hypotheses and to accomplish the proposed objectives as they relate to the cardiovascular system, patients will be evaluated for the following cardiovascular abnormalities:

1. Altered myocardial function
2. Altered chamber size and wall thickness
3. Cardiac valvular insufficiency
4. Endocarditis, active or healed
5. Pericardial disease
6. Structural heart disease
7. Tumors and masses

The tests most likely to detect and monitor progression of cardiac complications are listed as follows:

Table 17

Tests to Monitor Progression of Cardiac Complications

Complication	Physical Exam	Echo Doppler	ECG	Holter
Altered myocardial function	X	X		X
Altered chamber size and wall thickness		X	X	
Cardiac valvular insufficiency	X	X		
Endocarditis, active or healed	X	X		
Arrythmia	X		X	X
Pericardial disease	X	X	X	
Structural heart disease	X	X	X	X
Tumors and Masses	X	X	X	X

Infants and children with symptomatic vertically-transmitted HIV infection on enrollment, (Group I) are to undergo cardiac evaluation according to Table 18. This dynamic population has been noted to exhibit marked changes of left ventricular performance, afterload or contractility on serial echocardiography, usually related to clinical events such as respiratory failure, infections or

severe anemia (Lipshultz, Chanock, et al, 1989; Lipshultz, Sanders, et al, 1989A, 1989B). Abnormally elevated left ventricular mass, determined by echocardiography, demonstrated further serial increases in 73% of children with HIV infection (Lipshultz, Sanders et al, 1989C). This abnormal increase in left ventricular mass was more common in children with symptomatic HIV infection. Since changes in left ventricular function and mass occur frequently and may relate to clinical disease, an echocardiographic evaluation and a corresponding cardiac history and physical examination will be serially performed at 4 month intervals. A wide variety of conduction defects, voltage abnormalities compatible with chamber enlargement and dysrhythmias were noted by surface electrocardiography and holter monitoring in 93% of pediatric HIV patients (Lipshultz, Chanock et al, 1989). Serial electrocardiography has demonstrated progressive or new findings in 84% of these patients (Lipshultz, Chanock, et al 1989). Although electrocardiographic abnormalities were not related to HIV disease status, high grade rhythm disturbances were more common in patients with hyperdynamic left ventricular function (Lipshultz, Chanock, et al 1989; Lipshultz, Sanders et al, 1989A, 1989B). Hyperdynamic left ventricular function was observed in patients at all stages of HIV infection and was related to abnormal autonomic function in many cases (Lipshultz, Chanock, et al 1989; Lipshultz, Sanders et al, 1989A, 1989B). Therefore, the electrocardiogram and Holter monitor study are scheduled at yearly intervals. Cardiac catheterization with biopsy is performed as clinically warranted. Chest x-rays are utilized to assess the cardiac silhouette, pulmonary vascularity and upper airway obstruction (tonsillar or adenoidal hypertrophy) if possible with the frequency dictated by the pulmonary protocol. Molecular, histologic and gross anatomic pathologic assessment of endomyocardial biopsy and autopsy material are analyzed as available according to the pathology protocol.

Neonates born to HIV seropositive mothers and enrolled by 28 days of life (Group II) are to undergo cardiac evaluation according to Tables 19, 20 and 21. These children are followed to the age of 6 months before undergoing reclassification based on HIV culture and cardiac abnormalities as 1) Group IIa (HIV positive children-Table 19), 2) Group IIb - No Cardiac Abnormality (HIV negative children with normal cardiac studies - Table 20), or 3) Group IIb - Cardiac Abnormality (HIV negative children with cardiac abnormalities - (Table 21). The frequency, justification and rationale for performing cardiac evaluation in Group IIa is identical to that noted above for Group I children. In Group IIb, children without cardiac abnormalities undergo cardiac history, physical examination and echocardiograph at yearly intervals (Table 20), whereas Group IIb children with cardiac abnormality have these procedures done at six month intervals (Table 21). This group of control patients (HIV negative with no cardiac abnormalities) provides the necessary reference data required for analysis of study patients, as well as providing validation of preexisting echocardiographic control data, at this less frequent follow-up interval. Any patient following Table 20 who subsequently develops cardiac abnormalities will then be followed on Table 21 which provides the frequency of cardiac studies that all other patients with cardiac abnormalities will adhere to in this study.

FIGURE 3

SCHEMA FOR POSTNATAL CARDIAC STUDIES

GROUP I

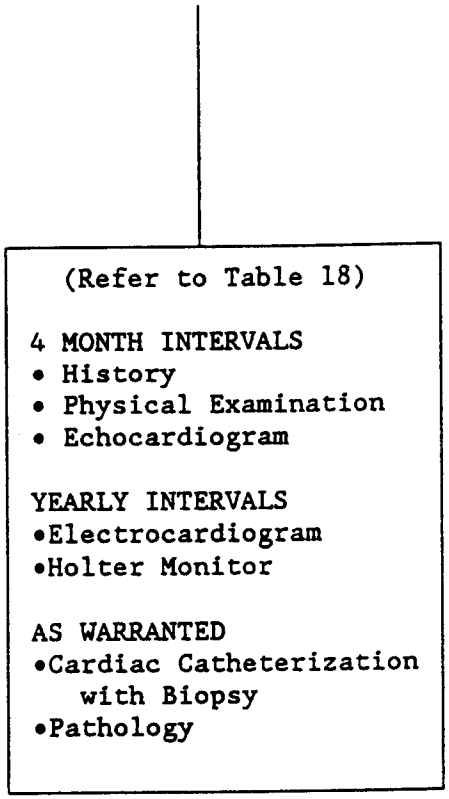


FIGURE 4

SCHEMA FOR POSTNATAL CARDIAC STUDIES

GROUP II: NEONATES BORN TO HIV SEROPOSITIVE MOTHERS

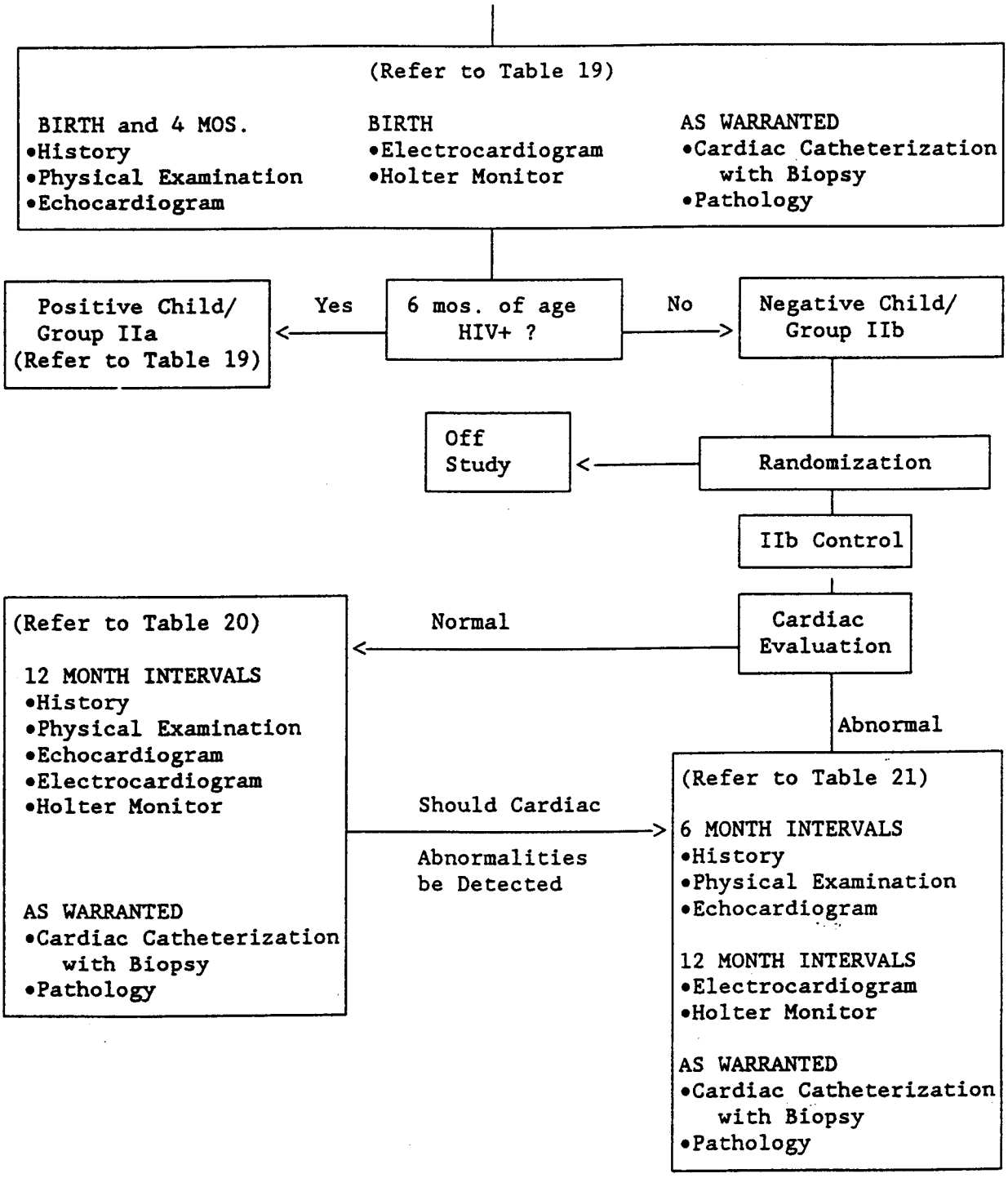


Table 18

Schedule of Cardiac Studies for Group I

Months on Study (1)	Cardiac History and Physical Examination	EKG	Holter Monitor	Echocardiogram	Catheterization with biopsy	Chest X-ray	Pathology - Molecular - Histologic - Gross Anatomic
0	X	X	X	X	As clinically	Per Pulmonary	Bx as clinically
4	X			X	warranted	Protocol	warranted
8	X			X			
12	X	X	X	X			
16	X			X			Postmortem
20	X			X			when available
24	X	X	X	X			
28	X			X			
32	X			X			
36	X	X	X	X			
40	X			X			
44	X			X			
48	X	X	X	X			
52	X			X			
56	X			X			
60	X	X	X	X			
64	X			X			
68	X			X			
72	X	X	X	X			
76	X			X			

(Rev. 6/10/93)

Table 19

Schedule of Cardiac Studies for Group IIa

Months on Study (1)	Cardiac History and Physical Examination	EKG	Holter Monitor	Echocardiogram	Catheterization with biopsy	Chest X-ray	Pathology - Molecular - Histologic - Gross Anatomic
0	X	X	X	X	As clinically	Per Pulmonary	Bx as clinically
4	X			X	warranted	Protocol	warranted
8	X			X			
12	X	X	X	X			
16	X			X			Postmortem
20	X			X			when available
24	X	X	X	X			
28	X			X			
32	X			X			
36	X	X	X	X			
40	X			X			
44	X			X			
48	X	X	X	X			
52	X			X			
56	X			X			
60	X	X	X	X			
64	X			X			
68	X			X			
72	X	X	X	X			
76	X			X			

Table 20
Schedule of Cardiac Studies for Group IIb Controls

Months on Study	Cardiac History and Physical Examination	EKG	Holter Monitor	Echocardiogram	Catheterization with biopsy	Chest X-ray	Pathology - Molecular - Histologic - Gross Anatomic
0	X	X	X	X	As clinically	Per Pulmonary	Bx as clinically
4	X			X	warranted	Protocol	warranted
12	X	X	X	X			
18	X			X			Postmortem
24	X	X	X	X			when available
30	X			X			
36	X	X	X	X			
42	X			X			
48	X	X	X	X			
54	X			X			
60	X	X	X	X			
66	X			X			
72	X	X	X	X			
78	X			X			

BLANK PAGE

5.3.3 Postnatal Echocardiography

The potential adverse effects of AIDS on myocardial contractile state have been examined in a preliminary fashion by several investigators. In some patients, decreased systolic function has been noted. However, these studies have invariably employed ejection-phase indices of ventricular function (such as ejection fraction) which are known to be highly dependent on cardiac loading conditions. The complex interaction of the cardiocirculatory system with neuroendocrine and renal factors which directly or indirectly influence cardiac load and contractile state is most likely to be disturbed in a multisystem disease state such as AIDS. Thus, recognition of the functional consequences of direct myocardial injury necessitates utilization of methods capable of discriminating between altered loading conditions and depressed contractility. In addition, due to the rapid changes in clinical status which are typical of this disease it must be possible to frequently repeat this evaluation, a condition requiring the use of noninvasive methodology.

Building on a series of observations in cardiac muscle preparations and in the isolated heart, the end-systolic force-length relation (Emax) has been shown to be both sensitive to contractile state and independent of loading conditions. This relation is linear within the physiologically relevant range, although deviations from linearity are noted at the extremes of myocardial length and load. Of particular relevance, the end-systolic force-length relationship is independent of events earlier in the cardiac cycle. That is, in spite of variations in end-diastolic fiber length and variable ejection load during early and mid-ejection, the end systolic length is determined exclusively by end-systolic force and contractile state. Thus, end-systolic wall force (which can be quantified as the end-systolic wall stress, ESS) represents the measure of afterload which determines the extent of myocardial fiber shortening, and is therefore the measure of afterload which is most relevant to the quantitation of myocardial performance. Wall stress is determined by ventricular dimension, wall thickness, and pressure. Colan, et al have developed noninvasive methods for determining wall stress as a measure of afterload and validated these against invasive standards (Colan et al, 1985; Colan, Borow, MacPherson, et al, 1984; Colan et al, 1983).

Ejection fraction (or percent fractional shortening, %FS) is known to be independent on ventricular afterload. When preload is constant, the relation of %FS to ESS (ESS-%FS) is inversely linear and sensitive to contractile state (Colan, Borow et al, 1984). Several authors have employed ESS-%FS as an index of contractile state in various disease states. However, the preload-dependence of %FS invalidates this index as a load-independent measure of contractility under conditions of variable preload. In contrast, the mean velocity of shortening (VCF) is an afterload-dependent index of contractile state which is independent of preload (Colan, Neumann et al, 1984). When VCF is normalized to a heart rate of 60 by division by the square root of the R-R interval, the rate-adjusted velocity of shortening (VCFc) is also independent of heart rate. The relation of VCFc to ESS (ESS-VCFc) has been shown to be an index of contractility which is independent of preload while incorporating afterload. The clinical applicability of this index has now been demonstrated in a number of studies (Colan, Sanders, Borow, et al, 1987; Borow et al, 1985; Colan, Sanders, Ingelfinger et al, 1987). Finally, since ESS-VCFc is determined by contractility

alone whereas ESS-%FS is dependent on contractility and preload, differences in these two indices are secondary to altered preload, thereby providing a functional index of preload. Each of these parameters (ESS, VCFc, %FS) can be accurately and reproducibly measured using totally noninvasive methodology.

These indices have been applied to a number of clinical situations. In children and young adults with end-stage renal disease, Colan et al, were able to distinguish the effect on ventricular performance of alterations in afterload, preload and contractility due to dialysis and transplantation (Colan, Sanders, Inglefinger, et al, 1987). Elite athletes with physiologic hypertrophy were noted to have marked changes in loading conditions with secondary alteration of systolic performance but normal contractility, effects which previous investigators had incorrectly ascribed to altered contractile state (Colan, Sanders, Borow et al, 1987). These methods have also been successfully employed in studies concerning the variable inotropic and vasodilatory effects of milrinone and amrinone. The utility of these indices in assessing contractile state and loading conditions in patients with acquired (doxorubicin cardiotoxicity) (Lipshultz, Colan et al, 1988); and congenital heart disease (ventricular septal defects, transposition of the great arteries) which are characterized by striking abnormalities in loading conditions have also been reported. Lipshultz et al, have detected a high incidence of abnormalities of loading conditions and contractility using these load-independent methods to assess ventricular function in infants and young children with HIV infection (Lipshultz et al, 1987; Lipshultz, Chanock, et al, 1989; Lipshultz, Sanders, et al, 1989A, 1989B, 1989C). This patient population has further emphasized the utility of this technique in distinguishing primary myocardial involvement from the adverse effects of altered load.

DATA COLLECTION

Ultrasound Equipment Requirements:

A high resolution ultrasound scanner using a 3.5, 5.0 or 7.5 mHz transducer and equipped with pulsed Doppler, 2-D directed M-mode, ECG, pulse and phono channels will be used. Hard copy capability for simultaneous 2-D directed M-mode, phono, pulse, and ECG will be available either in the form of a high quality strip chart recorder or a large format page printer.

General 2-D and Doppler Exam:

This portion of the exam is designed to detect masses associated with the heart and pericardial effusion, to evaluate valve function, and to evaluate qualitatively regional left ventricular function. The 2-D echo and Doppler exams are recorded on 1/2" VHS video cassette tape for subsequent review. The heart is scanned in parasternal long and short axis views and apical 2 and 4 chamber views. Subxiphoid long and short axis views are used where possible. Valve morphology and function is assessed by imaging and pulsed and/or color Doppler. Doppler interrogation of the atrioventricular valves is performed in apical and parasternal long axis views with attention directed to both the atrial and ventricular aspects of the valve. Semilunar valves are examined in parasternal long and short axis views and apical views, angling anteriorly to include the

pulmonary valve where possible. The subxiphoid coronal and short axis views provide an alternative window for the pulmonary valve. Again attention is directed to both aspects of the valves. Left ventricular regional wall motion is evaluated by scanning in parasternal long and short axis and apical 2 and 4 chamber views. Subxiphoid long and short axis views provide an alternative window. Particular attention will be directed toward obtaining the long axis dimension of the left ventricle with clear visualization of endocardium.

Left Ventricular Function Exam:

This part of the exam is designed to provide the physiologic data needed to calculate indices of ventricular function and to assess left ventricular loading conditions. The quality of the recording is highly dependent on the level of cooperation of the patient. It is uncommon for children below about 3 years of age to be able to cooperate sufficiently. It is usually necessary to sedate such patients.

High speed (100 mm/sec) hard copy of 2-D echo directed M-mode recordings of the left ventricular minor axis are obtained simultaneously with recordings of the electrocardiogram, phonocardiogram, indirect carotid or axillary pulse tracing, and peripheral blood pressure. Blood pressure is obtained using a Dinamap 845 Vital Signs Monitor.

Videotape and stripchart or page print recordings and blood pressure data will be sent to a central analysis site (The Children's Hospital, Boston) for analysis of LV size, function, loading conditions and contractility as described in the following paragraphs. The central analysis site has previous experience with multicenter use of this method of analysis. The central analysis site has documented that single center computer analysis dramatically reduces interobserver variability of analysis, in addition to reducing the equipment costs. The central analysis site has an extensive preexisting set of normative data for comparative purposes in infants and children of ages similar to those to be enrolled in this study.

DATA ANALYSIS

2-D and Doppler Echocardiogram:

The 2-D echo and Doppler exam are reviewed for masses and effusion. Valve morphology and function is recorded. Qualitative assessment of the LV shape and regional function are made, especially in short axis views. If there is uniform function and the LV is circular in cross-section, then formal evaluation of LV function (described below) is performed.

LV Size, Function, Loading Conditions, and Contractility:

The indirect carotid pulse tracing as well as the LV echocardiogram, including the endocardial border of the septum and the endocardial and epicardial borders of the LV posterior wall are hand digitized on a microcomputer based

digitizing station using custom software. This system is programmed to adjust the sampling rate to 200 Hz which is adequate to obtain at least 50 non-aliased harmonics at heart rates less than 120 beats per minute. The q wave of the ECG complexes bounding the cycles to be used as well as the onset of A2 and the onset of the carotid upstroke and the dicrotic notch are identified. After data input, the pulse transmission delay is corrected by electronically aligning the dicrotic notch on the pulse tracing with the first high-frequency component of the aortic valve closure sound. From the digitized data the following instantaneous measurements are obtained by averaging 3 to 6 cardiac cycles: (1) pressure during LV ejection, calculated by assignment of diastolic pressure to the minimum and systolic pressure to the maximum of the pulse trace and calculation of intervening values by linear interpolation; (2) LV internal diameter; (3) LV posterior wall thickness; and (4) LV meridional wall stress, calculated throughout ejection according to Mirsky:

$$WSm = \frac{(P)\ (D)\ (1.35)}{(h)\ [1 + (H/D)]\quad (4)}$$

where WSm is meridional wall stress in g/cm^2 , P is pressure in mm Hg, D is dimension in cm, h is the wall thickness in cm, and 1.35 is the conversion factor from mm Hg to g/cm^2.

From the continuous data, end diastolic values for dimension (EDD) and wall thickness (EDh) are taken at the time of maximum LV dimension and end-systolic values for dimension (ESD), wall thickness (ESh), blood pressure (ESP), and meridional wall stress (ESSm) are determined at the time of aortic valve closure (first high frequency component of the second heart sound). In subjects with no significant LV outflow obstruction by Doppler echocardiography, peak systolic meridional wall stress (PSSm) is calculated as the peak value for ejection meridional wall stress. Left ventricular ejection time is measured from the pulse tracing and adjusted to a heart rate of 60 beats/min by dividing the square root of the R-R interval on electrocardiogram. The fractional wall thickening (FWT) is calculated as (EDh - ESh/EDh). The LV percent fractional shortening (%FS) is calculated as (EDD-ESD)/EDD. The rate adjusted mean velocity of shortening (VCFc) is calculated as %FS divided by rate-adjusted ejection time.

Two dimensional echocardiographic measurements are performed on a video image capture and analysis workstation. After appropriate images of the LV in the transverse plane from the apex are digitized, the end diastolic frame is identified as the first frame after mitral valve closure and the end systolic frame is identified as the frame immediately preceding mitral valve opening. The long axis dimension is measured from the apical endocardium to the plane of the mitral valve annulus at end diastole and end systole. Peak and end systolic circumferential wall stress (WSc) are then calculated from the long axis dimension (D), short axis dimension (R), pressure (P), and wall thickness (h) according to the method of Mirsky:

$$WSc = \left[\frac{(P)\ (R)\ (1.35)}{(h)}\right]\ \left[1 - \frac{(R)\ (R/D)^2}{(2R + h)}\right]$$

Since peak wall stress occurs very early in systole and the total change in LV long axis length is small, the end diastolic long axis length is used to estimate peak circumferential stress while the end systolic length is used for end systolic circumferential stress. The short axis dimension and LV pressure coincident with peak and end systolic meridional stress are used to calculate the peak and end systolic circumferential stress, respectively. The time course of development of meridional and circumferential stress has been shown to be similar.

These measurements are compared with values obtained in a cohort of 250 normal subjects aged 2 weeks to 20 years for recognition of ventricular dilation, hypertrophy or reduced wall thickness, excess afterload (ESS), and reduced ventricular systolic performance and contractility (Lipshultz, personal communication). The ventricular configuration, characterized by the long axis:short axis ratio and the ESSm:ESSc ratio is also compared with the normal range. The further analysis of indices of contractility and preload is then conducted identically for both circumferential and meridional stress as described below.

The relationship between VCFc and ESS has been previously shown to be an afterload-adjusted, preload-independent index of contractility. To facilitate comparison of patient groups, the position of the relation of ESS to VCFc for each patient is determined relative to the distribution of this index in normals and calculated as the stress-velocity index (SVI) = the number of standard deviations from the population mean. Thus, an SVI < -1.96 implies reduced contractility. In contrast, the relation of %FS to ESS is an afterload-adjusted index of contractility which is sensitive to alterations in pre-load. The ESS - %FS relation is correspondingly quantified as the stress shortening index (SSI) = the number of standard deviations from the population mean %FS for the given ESS. The relative magnitude of VCFc compared with %FS for any level of ESS is a measure of the preload status, reflecting the fact that fractional shortening is directly related to end-diastolic fiber stretch whereas VCFc is independent of preload. Differences between the ESS-VCFc relation and the ESS-%FS relation therefore reflect the functional consequences of altered preload, which is quantified as the functional preload index (FPI) = SSI - SVI. An FPI which is outside the range of the age-appropriate values will be interpreted to indicate abnormal preload.

Left ventricular mass is calculated using a modification of the formula of Devereux and Reichek (1977): Mass = $1.04[(D + 2h)^3-D^3]-14g$.

Right Ventricular Systolic Pressure:

Right ventricular systolic pressure is estimated by assessment of the interventricular septal position during late systole in subxiphoid or parasternal short-axis views (Lipshultz, Sanders, et al, 1988). Right ventricular pressure is estimated as 1) <1/2 systemic if septal curvature is convex toward the right ventricle; 2) between 1/2 systemic and systemic if the septum is straight; and 3) ≥ systemic if septal curvature is convex toward the left ventricle. If possible, right ventricular systolic pressure will also be

assessed by measurement of the peak velocity of the tricuspid regurgitant jet by continuous wave Doppler ultrasound as RV peak systolic pressure = (4 x V^2) + CVP where V = peak velocity of the TR jet and CVP = measured CVP or estimated CVP = 5 mm Hg.

5.3.4 Electrocardiography

A standard electrocardiogram (at least 12 leads) will be performed using an electrocardiograph that employs analog to digital conversion (e.g., Marquette, Hewlett-Packard). Temporal and voltage indices of rate, chamber size, QRS vector, T wave configuration, and rhythm will be obtained by conventional techniques.

5.3.5 Holter

A standard Holter recorder capable of recording two simultaneous leads will be used. These leads should include a lead II equivalent or MCL5, and an anterior chest lead such as MCL1. At lease 18 hours of interpretable data should be recorded. A maximum duration of any pause will be recorded. The rhythm will be interpreted by standard criteria, (Garson, 1983). The frequency of premature beats is defined as follows: infrequent (less than 10 per day) and moderate (10 per day one per hour), frequent (greater than 1 per hour). For any episodes of tachycardia, the number of episodes and the rate and the duration of the longest episode will be recorded.

5.3.6 Cardiac Catheterization and Endomyocardial Biopsy

Endomyocardial biopsy will be done for clinical indications. Morphologic correlates of cardiac functional abnormalities described in AIDS patients include lymphocytic myocarditis and/or lymphocytic infiltrates and nonspecific findings of ventricular dilatation, myocardial hypertrophy and fibrosis indicative of a dilated cardiomyopathy. Infectious agents (toxoplasma, cryptococcus, mycobacteria, CMV, EBV) have been identified in a few patients with lymphocytic myocarditis/pericarditis however, in most, a specific agent has not been found. The role of other cardiotrophic viruses, such as Coxsackie which is thought to cause a significant number of cases of myocarditis in immunocompetent patients, but often is not identified in the myocardium, is unclear. The HIV virus has not been identified in the myocardium and its role in the development of myocardial inflammation and/or myopathy remains to be established. Similarly, the pathogenetic mechanisms in the development of the dilated cardiomyopathy seen in these patients also remains to be determined. Although in most cases of non-HIV associated dilated cardiomyopathy, lymphocytic myocarditis is thought to be a significant etiologic factor, in the AIDS population there are many potential contributory factors which have been associated with cardiac dysfunction. In addition to the many opportunistic infections and frequent multi-organ system failure, these patients are often anemic and nutritionally deficient. Also, the role of pulmonary disease in the development of right ventricular dysfunction has yet to be established.

If clinical symptoms and signs of congestive heart failure occur in conjunction with abnormalities in systolic ventricular performance, patients will undergo right and left heart catheterization using standard techniques, at the discretion of each individual center. After hemodynamic measurements are completed, cineangiocardiograms will be performed in the right and left ventricles in orthogonal planes for measurement of ventricular volumes. If pulmonary vascular resistance is elevated, pulmonary arterial wedge angiography will be performed in the posterobasal segment artery of the right or left lower lobe, and vascular changes will be analyzed and quantified using the method of Rabinovitch et al, (1981). After hemodynamic measurements are completed endomyocardial biopsy will be performed using standard techniques.

Tissue Analysis

Analysis of endomyocardial biopsy samples will be performed in each Clinical Center. Biopsy fragments will be fixed for light microscopy, electron microscopy and frozen according to a standard protocol. The biopsy will be evaluated histologically for evidence of fibrosis, necrosis, inflammation, and other changes.

Viral identification by in situ hybridization or immunohistochemical methods of agents with the potential to cause myocarditis and/or myocardial dysfunction will be done when clinically warranted. In other cases tissue will be appropriately preserved such that these studies and others (immunohistochemical analysis of cellular markers, polymerase chain reaction, lymphocyte marker studies) can be done as ancillary studies.

5.3.7 Evaluation of Asymptomatic Patients

If any one of the following conditions are present on postnatal echocardiogram, ECG or Holter monitor, a cardiac assessment (Form 71) and cardiac physical examination (Form 79) will be performed by a pediatric cardiologist to determine the clinical cardiovascular status of the patient.

Echocardiogram findings requiring cardiac assessment and physical examination:

1. Fractional shortening < 20% or a change in fractional shortening >25%.
2. Pericardial effusion
    a. Localized or circumferential with 5–10 mm diameter
    b. Circumferential with > 11 mm maximal diameter
3. Valvular regurgitation
    a. Aortic regurgitation, jet diameter > 3 mm
    b. Pulmonic regurgitation, pathologic
    c. Mitral regurgitation, jet diameter > 3 mm
    d. Tricuspid regurgitation, jet diameter > 3 mm

4. Structural abnormalities
    a. Ventricular septal defect
    b. Atrial septal defect
    c. Pulmonary stenosis
    d. Patent ductus arteriosus after 2 months of age
    e. Other significant structural abnormalities

ECG and/or Holter monitor findings requiring cardiac assessment and physical examination:

1. 2nd or 3rd degree heart block on ECG or Holter monitor
2. Supraventricular tachycardia
3. Atrial fibrillation
4. Atrial flutter
5. Ventricular tachycardia
6. Ventricular fibrillation
7. "Q" wave > 0.35 seconds, any leads
8. ST depression > 2 mm, any lead

5.3.8 Evaluation of Patients with Possible Symptoms related to the Heart

The patients being studied are at a high risk of developing significant cardiac problems. Therefore, if any one of the following conditions are present in a symptomatic patient, a cardiac assessment and cardiac physical examination, to determine the cardiovascular status of the patient will be triggered.

1. Unexpected or unresponsive respiratory symptoms >7 days not thought to be due to lung disease
2. Persistent respiratory symptoms > 2 weeks in conjunction with documented pulmonary illness
3. Signs and/or symptoms of pericardial effusion or endocarditis
4. Signs and/or symptoms suggestive of congestive heart failure
5. Presence of frequent ectopy, unexplained "blue spells", unexplained seizures, unexplained syncopy or significant autonomic dysfunction

The cardiac physical examination is to be performed by a pediatric cardiologist. If cardiac disease cannot be excluded, then further cardiac evaluation should be considered.

The following modifiers have known association with HIV and may be seen in HIV-infected patients with pericaridal effusion, LV dysfunction or structural heart disease. These disorders can be detected, if present, by a P^2C^2 postnatal echocardiogram evaluation. It is recommended that the following modifiers be considered at the time of the examination, as they may help direct the course of the evaluation (Figure 8, page 78b).

- Severity of intercurrent illness warrants hospitalization
- Evidence of clinical co-infections (e.g. EBV, CMV, MAI) present in symptomatic patient
- Evidence of HIV encephalopathy
- CDC-defined AIDS or severe immune dysfunction
- Antiretroviral therapy > 1 year and/or interferon therapy
- Known selenium or carnitine deficiency
- Past history of LV dysfunction or dilation, structural abnormality or pericardial effusion on echocardiogram

The following modifiers have known association with HIV and may be seen in HIV-infected patients with ectopy. Ectopy is likely to be detected by P^2C^2 EKG and Holter monitor evaluations. It is recommended that these modifiers be considered at the time of the cardiac examination, as they may help direct the course of the evaluation (Figure 8, page 78b).

- Severity of intercurrent illness warrants hospitalization
- Evidence of clinical co-infections (e.g. EBV, CMV, MAI) present in symptomatic patient
- Evidence of HIV encephalopathy
- CDC-defined AIDS or severe immune dysfunction
- Therapy with IV pentamidine and/or ganciclovir
- Past history of LV dysfunction or dilation, structural abnormality or pericardial effusion on echocardiogram

5.4 Pathological Studies: Postmortem Examination of HIV-Infected Infants in Groups I and II

A number of infants and children in the study population will die each year. It is estimated that at least 50% of the P^2C^2 HIV study patients who die will come to autopsy. Postmortem examination will provide important morphologic correlation with the antemortem functional assessments and clinical indicators. Consent for autopsy will be obtained in the customary manner for each institution using their standard hospital autopsy consent form. In those cases in which permission is obtained, the autopsy will be done in a timely fashion, generally within 24 hours of the demise of the patient. If there are no restrictions made in the consent given, a complete autopsy will be performed. A standard protocol will be used to ensure that appropriate cultures are performed, and that appropriate tissue is sampled and preserved for light microscopy, electron microscopy, and other special studies that may be done as ancillary studies.

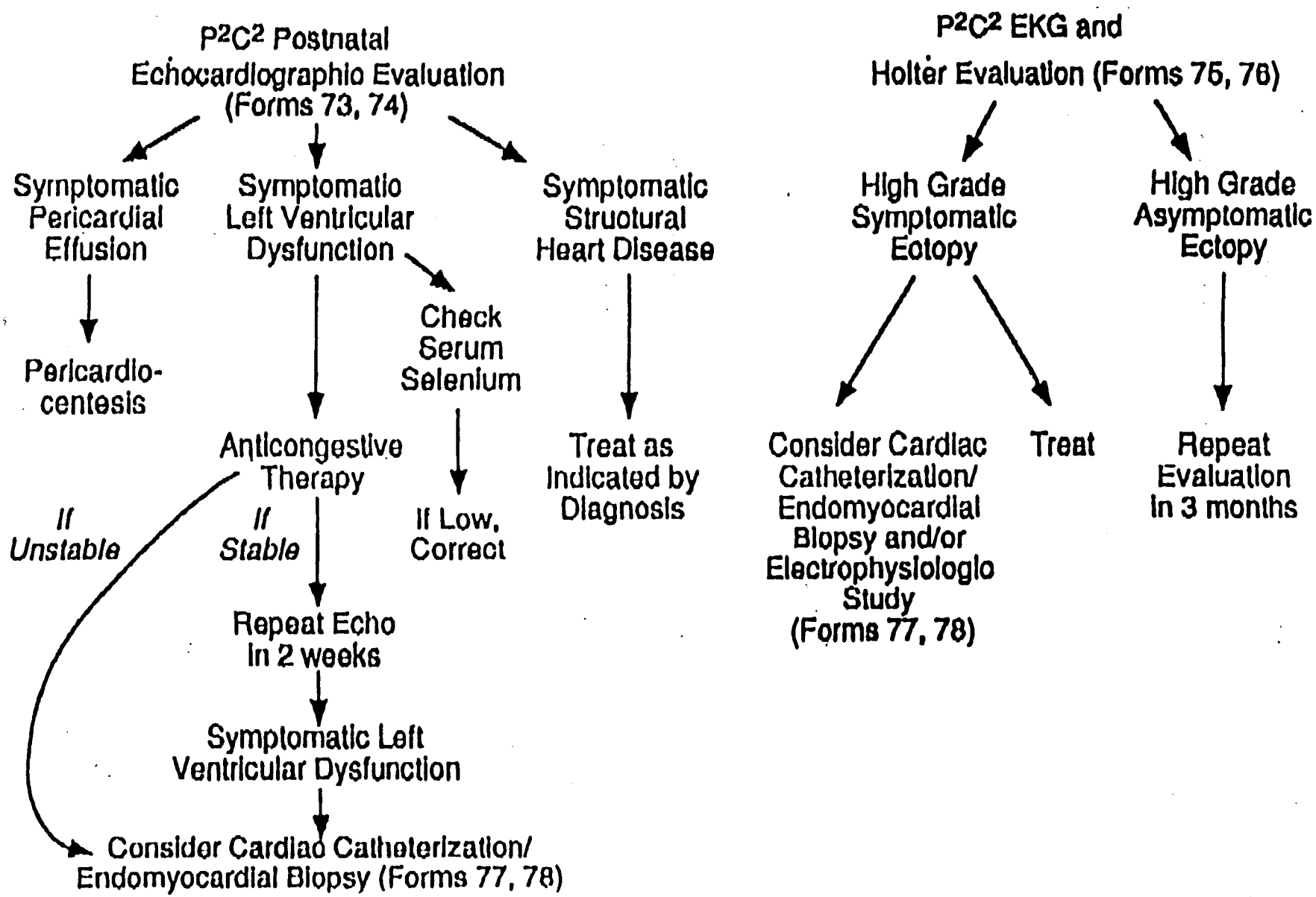
The lungs will be prepared in a standard fashion by inflation fixation so that they will be suitable for morphometric assessment for correlation with clinical studies of pulmonary function as an ancillary study.

The heart will be carefully examined for structural malformations, chamber dimensions, ventricular wall and valve measurements according to a standard protocol so that appropriate tissues are retained for examination and ancillary studies.

Lesions of small and medium sized arteries have been described in the brain, kidneys, lungs, spleen, skeletal muscle, thymus, and small intestine. The brain was consistently involved. Apparently, larger musculoelastic arteries are not affected; however, it is not clear which such vessels have been examined in published reports. No arterial lesion was identified on gross examination and none could be seen radiologically. In order to examine the incidence and extent of arterial involvement in pediatric AIDS, arteries from various sites will be systematically examined in addition to evaluation of intraparenchymal vascular branches within the various organs.

Figure 8

EVALUATION OF PATIENTS WITH POSSIBLE SYMPTOMS RELATED TO THE HEART



6. Distributed Data Entry and Data Management

Figures 5 and 6 diagram the computer communication network, flow of study data, respectively. Various components of this system are described in detail in the following sections.

6.1 Computer Systems

6.1.1 Hardware and Software at the Clinical Coordinating Center

Hardware

The Clinical Coordinating Center will have personal computers for the purpose of collecting and analyzing study data. The hardware set-up includes two (2) IBM PS/2 model 70 A21 computers, with memory (2MB) and hard disk space (120MB) deemed sufficient for the duration of the study. A 2400 baud Hayes modem for electronic data transfer and communications as well as printers (HP LaserJet Series II and an IBM Proprinter X24E for producing hard copies of output. Access to an IBM 3090 mainframe computer is available for sophisticated data analyses needs.

Software

The MS-DOS operating system will be utilized throughout the duration of the study. A database management system will be used for data collection and reporting. Carbon Copy Plus software will be utilized for the transmission of study data from the Clinical Centers and for troubleshooting. This software will provide remote site computer diagnosis and correction. SAS is available to CCC personnel, both on the PS/2 microcomputer and the IBM 3090 mainframe, for statistical analyses and report preparation. Other statistical software including BMDP are also available.

Additional software will be acquired and implemented and upgrades to currently specified software will be obtained, as necessary, for smooth operation of the study.

6.1.2 Hardware and Software at the Clinical Centers

Hardware

Each Clinical Center will be provided with their own personal computer for the purpose of collecting study data. The hardware set-up will include an IBM PS/2 model 70 E61 computer, with memory (1MB) and hard disk space (60MB) deemed sufficient for the duration of the study. A 2400 baud Hayes compatible modem for electronic data transfer and communications, as well as a printer for producing hard copies of outputs, will be provided.

The system including hardware and software will be delivered by the CCC to the Clinical Centers "turn-key" ready.

Software

The MS-DOS operating system will be utilized throughout the duration of the study. Carbon Copy Plus software will be used for the transmission of study data to the CCC. A database management system identical to that, in use at the CCC, will be included for data collection and reporting.

Additional software will be acquired and implemented and upgrades to currently specified software will be obtained, as necessary, in order to secure smooth operation of the study.

6.2 Communication

Figure 5 illustrates the main components of the communication network.

6.2.1 Data Transmission

Data transmission from the Clinical Center to the CCC will occur every night and will be initiated by the CCC. To ensure confidentiality and safety of study data, neither the Clinical Centers nor other outside persons will have the potential to call the CCC for the purpose of transferring data. Software will require that the appropriate password be sent to the Clinical Center computer from the CCC computer, before transmission will begin. These passwords will be controlled by the CCC and thus only the CCC will have the capability of initiating data transfer from clinical center microcomputers. Data transmission will occur in the early morning hours when phone rates are at their lowest. It will be automated such that the data transfer can occur in an unattended mode.

Data transfers will include only data that have not previously been transferred to the CCC. Hence, only newly entered data or data that are the response to a CCC query will be transferred. Data extraction programs, provided by the CCC, will create the appropriate subset of data that will be transferred to the CCC. When no new data are available for transmission, specific codes will allow this case to be distinguished from possible software failures.

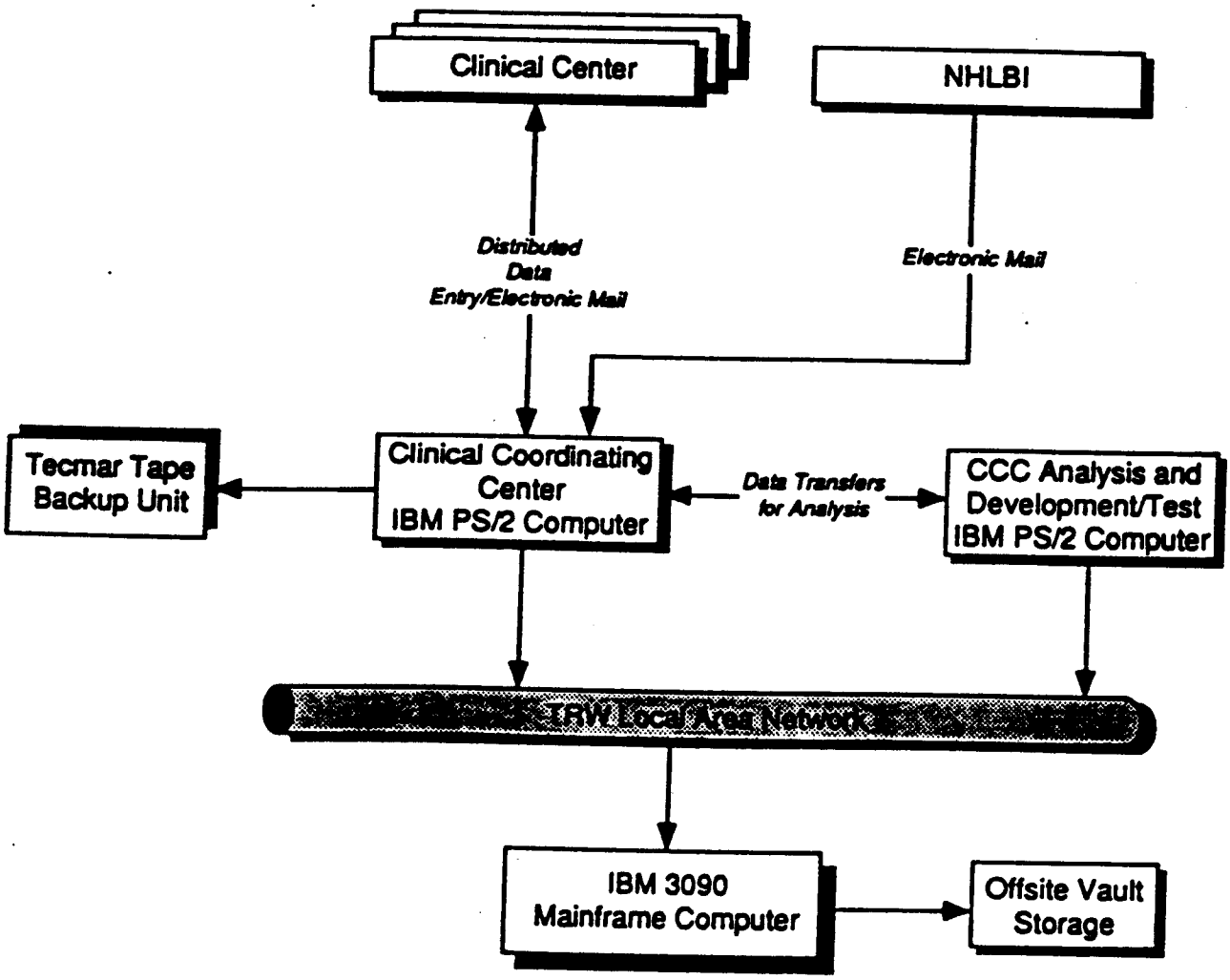
6.2.2 Electronic Mail

Electronic mail capabilities will be provided for the purpose of passing study information between the Clinical Centers, NHLBI, and the Clinical Coordinating Center. Reports and information pertaining to patient data and study issues will be electronically transmitted, each night, to each Clinical Center, as required. Similarly, messages or information that the Clinical Centers need to send to the CCC will be electronically transmitted to the CCC, as necessary.

In addition, each Clinical Center will be able to communicate with each other or NHLBI by this electronic massaging methodology, thus allowing a 24 hour maximum "mail" delivery time. All mail will be routed through the CCC and then distributed to each of the Clinical Centers.

Note that all data communication connections will be established by the CCC calling the Clinical Center. The CCC will then send an appropriate password to the Clinical Center's computer and only upon validation of this password will any information exchange proceed. Centralizing the electronic mail functions in this way gives the CCC better control over the flow of information between the Clinical Centers and the CCC as well as information exchange between Clinical Centers.

Figure 5
Computer Communications Network



6.3 Data Editing and Integrity

6.3.1 Data Editing at the Clinical Centers

Data editing and data consistency checking will occur at the Clinical Center, to the extent possible, and at the CCC. Standard editing techniques will be employed. Numeric data will only be allowed in numeric fields. Dates will be checked for validity as well as chronological correctness. Fields requiring computation based on previously entered data will be automatically computed and stored.

In addition to interactive editing, re-key verification will be employed. This will require all data to be entered into the computer twice. Once in the standard entry mode and then a second time in a verify mode. Any inconsistencies (e.g., data which does not match the previously entered data) will cause a warning to be sent to the data entry operator. It will be up to the individual doing the key entry to determine which of the discrepant values is correct and indicate this to the database system. Implementation of such a double entry or re-key verification system will reduce the amount of erroneous data received by the CCC due to key entry errors. A database of errors will be maintained in order to validate the necessity to re-key data.

As much data editing as is possible will be performed at the Clinical Center at data entry time. However, some data validation and consistency checking will only be able to be performed at the CCC. Checking consistency between currently entered data and data previously sent to the CCC is an example of such a validation. Data that fails to meet such consistency checks will be rejected from the study database and placed in a query database until a resolution over the data item in question has been established.

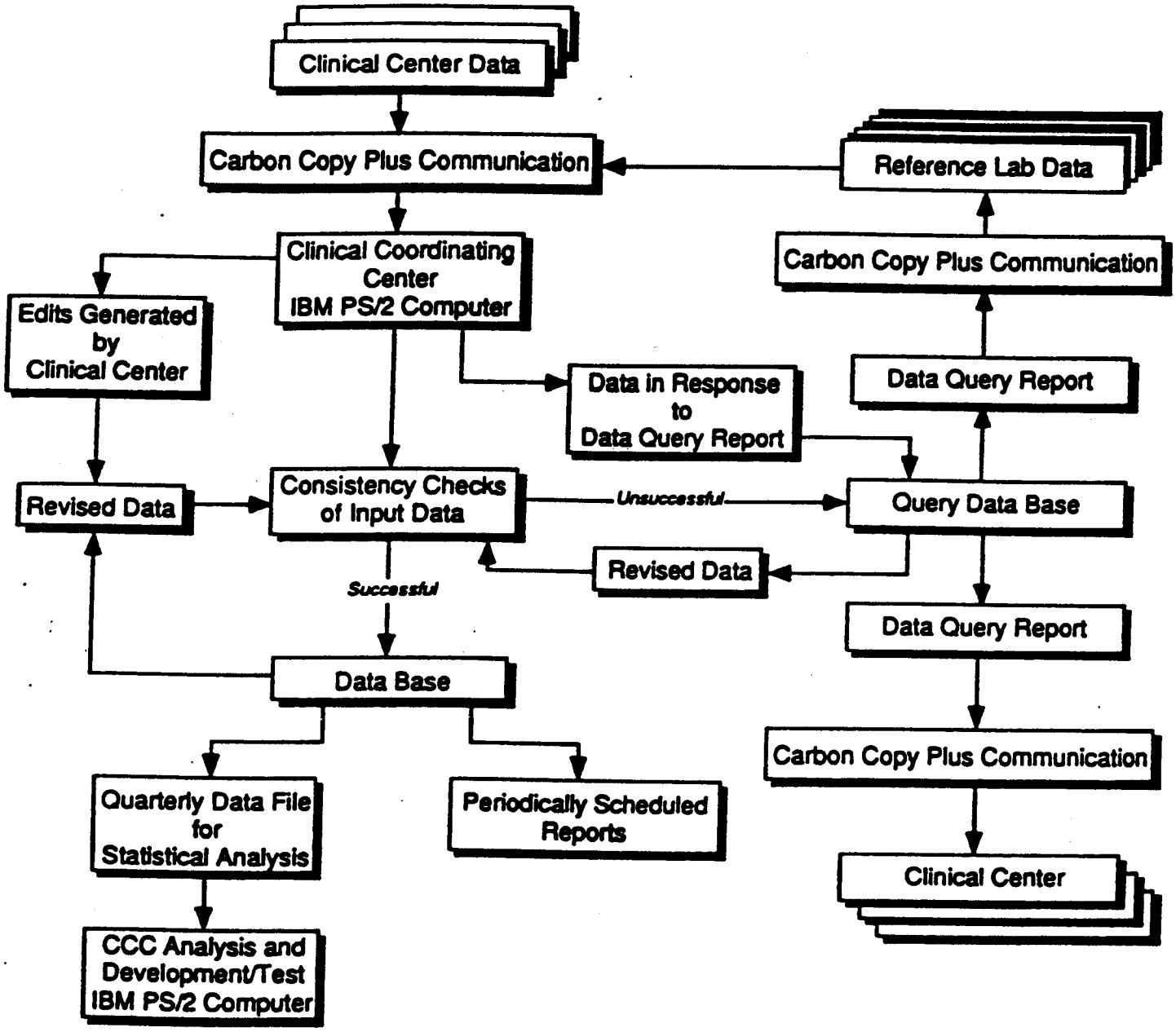
As diagramed in Figure 6, the microcomputer will edit data following transmission. If no inconsistencies are detected, the data are added to the main database. If however, there are inconsistencies in the data, the information is retained in the query database and is not added to the main study database until errors are resolved. These errors will be summarized in Data Query Reports and be used to revise the information in the query database. Data received from Reference Laboratories will also be subject to consistency checks and Data Query Reports. Query Reports will be generated and returned to the laboratories for correction.

The edits will locate errors within each form and relational checks will be made against data previously collected on a given patient.

Upon reviewing reports of information provided to the CCC, a Clinical Center might identify data which, although passing the consistency checks, is in error. The transfer of these edits to the CCC will result in the original data being extracted from the main database, revised and subjected again to the original consistency checks.

Out-of-Range Data forms submitted by the Clinical Centers will be reviewed by the CCC staff. The data files on the PS/2 will be modified accordingly with the appropriate audit trail completed.

Figure 6
Data Flow



6.3.2 Data Processing at the Clinical Coordinating Center

After data transfer, data query reports generated from previously submitted data and messages will be downloaded to the Clinical Center's computer. Following these data transfers, the consistency checks for the input data will be automatically performed and reports generated. Extensive computer edits and data verification will take place following transmission. Valid data will be stored in the main database, inconsistencies will be retained in a query database.

6.3.3 Resolution of Data Discrepancies

Discrepancies which are identified, will be relayed to the Clinical Center via nightly transfers as Data Query Reports. The Clinical Center Data Manager will respond by checking the original forms for inconsistency, checking other sources to determine the correction, modifying the original (paper) form, and entering corrections via forms. The data will again be subjected to consistency checks.

In all phases of the editing procedure, an audit trail will be developed such that it will be possible to identify all changes that have been made in the data files and when these changes occurred. It will be possible to reconstruct the database at any point in time. A separate Quality Control audit record will be created in the database. This record will indicate when and which specific patient data information has been added, updated or deleted from the database.

Data corrections will not be made over the phone. Written documentation of changes will be available.

Follow-up procedures will be established to assure that all errors are corrected. A Missing Corrections Report will consist of queries which have been identified and have not yet been corrected by the Clinical Centers. It will highlight corrections which are over 14 days delinquent. A batch program will be run by the CCC staff on the first day of the month which will produce monthly reports to be sent to the Clinical Centers. The morning after the batch program is run, the CCC staff will send Missing Correction Reports to the Clinical Centers via electronic mail. At the Clinical Center, the Data Manager will sign on and get a hard copy of the Missing Corrections Report for his or her reference. The Data Manager should then gather the appropriate information to respond to the queries outstanding and follow the instructions found in the Data Query Report for correcting invalid data.

6.3.4 Quality Control of Study Data

In addition to the editing and consistency checks described above, a subset of data will randomly be selected and reviewed to identify systematic errors, document database error rate and evaluate entry at Clinical Centers. Clinical Centers and reference laboratories will submit hard copies (forms) of all data electronically communicated to the CCC. Approximately 5% of these forms will randomly be selected and entered at the CCC. These data will be compared to that

previously received and any inconsistencies noted and tabulated. These process will be closely monitored, and if after six months it is not cost effective, the system will be modified.

6.3.5 Data Integrity

It is essential to maintain proper data integrity of the study database and also insure that data analyses are based on the central CCC study database. Since multiple sites have access to database management software the potential exists for individual centers to maintain their own private database systems and create reports relating to their particular center. This type of activity is of value if the results of the reports are maintained within the confines of the individual center. Sharing data of this nature is prohibited under the contractual agreements and would violate the P^2C^2 HIV "publication" policy. The CCC and the NIH will only acknowledge reports and analyses based on data from the central CCC database. The CCC database must and will be looked upon as the study database.

6.4 Data Backup and Recovery

6.4.1 Backup at the Clinical Centers

Clinical Center personnel will be trained in proper backup procedures for their PS/2 computer. Full backups of Clinical Center computers will take place on a monthly basis and will be retained for a minimum of 8 weeks.

A backup, to floppy disk, of the data subset that will be transmitted to the CCC will be made automatically each night under the data transfer programs control. It will, however, be the responsibility of Clinical Center personnel to appropriately label and store these backups. Such diskettes will be handled in a manner consistent with proper computer diskette care.

6.4.2 Backup at the Clinical Coordinating Center

The Clinical Coordinating Center will incorporate several backup procedures as part of its standard operating procedures.

First, all study data received from each individual Clinical Center will be retained in its original unedited, uncorrected form prior to being loaded to the main study database. Hence, a copy of all files received from each Clinical Center will be copied to a separate subdirectory on the CCC central computer. This subdirectory will be backed up on a daily basis to insure that the original data will not be lost.

Second, the study database will be backed up to tape on a weekly basis and retained for a period of 8 weeks.

Third, a copy of the database will be placed on the CCC second PS/2 computer on a weekly basis. This will minimize crossover time in the event the CCC main data collection system should experience a hardware failure.

Fourth, a full backup of both data and programs in the CCC computers will be done on a monthly basis. This will utilize the production backup procedure on the 3090 mainframe (Figure 5). The tapes containing these backups will be retained in offsite environmentally controlled vault storage.

The net result of these extensive and redundant backup procedures will be the minimization of downtime and complete database recovery should a catastrophic event occur.

6.5 Confidentiality

Confidentiality of data collected by the Clinical Centers and the Clinical Coordinating Center is a major priority of this multicenter study. This issue is particularly sensitive since the study involves subjects being diagnosed with the HIV infection or who are at high risk for developing the infection.

Particular care must be taken to ensure the confidentiality of participants in this study, to the extent possible, under applicable laws. All persons engaged in the collection, handling or dissemination of patient data shall be specifically informed of their responsibility to protect patient data and of the penalty for violation of this trust. Confidentiality statements will be signed by all employees and data users. Unauthorized acquisition, release, and/or discussion of any information to persons not in association with the contract is strictly prohibited, unless otherwise authorized by the NHLBI.

The collection of patient data, whether by interview, observation or review of documents, shall be conducted in a setting which offers maximum privacy and protects information from unauthorized individuals. Data will be housed in a secure area and will not be left unattended in areas accessible to unauthorized individuals. All patient information will be kept in locked files and will be accessible only to authorized personnel. Offices will be locked in off duty hours.

All correspondence (e.g., letters, forms, tapes, reports, etc.) containing patient information, mailed to and from the CCC, will be marked "CONFIDENTIAL". Only authorized personnel will be allowed to handle such correspondence. Requests for data on patients via telephone will require proper identification and verification to assure that the requesting party is entitled to receive such information. A record of the request will be maintained. Any destruction of correspondence containing patient data shall be according to an approved facility and will be accomplished by controlled incineration, shredding or other acceptable means of document disintegration. Data stored on electronic media will be bulk erased.

All data will be identified by code only. All data collection forms will utilize the code rather than a subjects name. Some data on each subject will be maintained for the Clinical Center to follow the patient but this will not be reported to the Clinical Coordinating Center.

All computers and data storage media will be kept in secured areas. Only authorized study personnel will have access to such areas. All disk files, backed up weekly at the CCC, will be stored in a locked cabinet. Disk files backed up monthly, will be stored in a vault in a remote location. This vault is especially secure and climate controlled to protect and preserve magnetically stored information.

6.6 Training of Clinical Center Data Management Personnel

6.6.1 Introduction

The success of this study largely relies on the quality of data that is collected. To ensure that quality data is collected, the Coordinating Center will require that all Clinical Center Data Managers be trained and certified prior to the enrollment of any patient into the study. Additional training sessions will also occur during the course of the study to certify new personnel and/or to comply with changes in the protocol.

6.6.2 Teaching Methods

6.6.2.1 Training Sessions

Initial Training Session

A central training session will be held at the Cleveland Clinic Foundation (proposed date April of 1990). Training sessions will deal with the protocol, data collection, entry, transmission, discrepancy resolution, and general data management. The protocol, manual of operations, forms, and other materials will be distributed to the appropriate Clinical Center personnel prior to the training session.

Retraining Sessions

Data Management staff at the Clinical Centers, who demonstrate during the course of the study expertise in the use of these systems, will be used as a resource for assistance in training other personnel. They will assist in training sessions for Data Managers who are less than proficient and in training held for new personnel.

6.6.2.2 Phone Calls

Throughout the data collection period, the CCC staff will be available on a daily basis, by phone, to respond to ad hoc questions from the Clinical Center

personnel. Conference calls can also be arranged between the Clinical Center, CCC and Program Office when needed.

6.6.2.3 Monthly Reports

A monthly report will be prepared by the CCC to keep all study personnel and NHLBI informed of various aspects of the study. The report will contain announcements of meetings, number of subjects registered on the protocol, changes in data collection, forms, and/or amendments, etc.

6.6.3 Certification of Data Managers

At the completion of the training sessions, the CCC will certify Clinical Center personnel who are able to complete the data collection forms and enter the information in the distributed data entry system in the manner prescribed. In order to meet certification, Clinical Center personnel must demonstrate proficiency in forms completion, data entry, setting the microcomputer for data transmission, and responding to query data reports. Records documenting the data management staff who were trained and the results of their training will be maintained at the CCC.

6.6.3.1 Forms Completion Certification

In order to meet the "forms completion" certification criterion, the Data Manager must successfully complete a forms completion quiz developed by the CCC.

6.6.3.2 Data Entry Certification

In order to meet the "data entry" certification criterion, the Data Manager must successfully enter a form which was completed by the CCC staff. They may use only the instructions in the Manual of Operations to complete the data entry. The data file created by this entry must match a file created by the CCC staff for at least 90% of the data fields.

6.6.3.3 Data Transmission Certification

In order to meet the "data transmission" certification criterion, the Data Manager must successfully complete the procedures necessary to initialize the transmission files and leave the microcomputer in a configuration so that automatic transfer of data can be completed when initiated by the CCC. They must also demonstrate proficiency in executing the software which will print the "electronic mail" messages for distribution.

6.6.3.4 Query Data Report Certification

In order to meet the "query data report" certification (error correction) criterion, the Data Manager must successfully respond to a series of queries created by the CCC staff. The responses are to be entered into the microcomputer and placed in a file which has been initiated for transmission to the CCC.

7. Data Analysis

7.1 Introduction

Since the sample for this study will not be a random sample from a general population, a description will be prepared of the baseline variables of the subjects enrolled into each of the Groups I and II: (I) Infants and children with symptomatic HIV-infection, (II) Newborn infants of mothers entered into the study in whom HIV antibody tests were positive during pregnancy. Group II will also be split into Groups IIa and IIb based upon the child's own confirmed HIV status. Such a description will help to characterize these subjects. The variables considered in such a description could be categorized as follows: (1) demographic characteristics, (2) mothers' medical history, (3) cardiac data, (4) pulmonary data, and (5) immunological and infectious disease data. The description will include medians and confidence intervals on means and proportions.

The general approach to the analysis will be one that concentrates on a better understanding of the "natural" history of pediatric HIV infection. Although some of the analyses to be performed for this study will involve tests of hypotheses, (e.g., comparison of HIV-positive and HIV-negative children), several of the objectives of the study relate to questions of estimation within groups. For example, estimation of the incidence of HIV-infection in Group II and of various complications in Groups I, IIa and IIb will be obtained as well as their associated confidence intervals. It is also important to keep in mind that many comparisons and confidence intervals are to be made. Therefore, multiple comparisons may yield a significant result due to chance alone. While testing hypotheses at the 1% significance level will help to adjust for this, the magnitude of differences will also be examined.

A problem that may arise with this population is incomplete data and timing of follow-up visits. Also, the exact time points when events (HIV infection, complications, etc.) occur may not be known. Primary outcome variables may be the development of: HIV infection in Group II, major complications such as left ventricular dysfunction, PCP and lymphoproliferative lung disease, and death (although the rate may be low given the relatively short follow-up period of the study). Survival (time-to-event) analysis will be the primary type of analysis to be used.

As stated in Section 4.3, a variety of efforts will be made to minimize the drop-out of subjects during the study. At least vital status of any subject lost-to-follow-up will be determined. Baseline and other known characteristics of subjects lost-to-follow-up will be compared to those not lost to determine whether there are any differences which could potentially bias inferences.

7.2 Statistical Methods

Specific statistical methods that will be used to evaluate each primary and secondary hypothesis or objective are described in this section.

The incidence, prevalence and recurrence rates of cardiac and lung complications (Objectives O1 and O3) will be summarized using confidence intervals on proportions. These rates will be determined at various time points (e.g., fetus, neonate and age 2.5 years); and will also be calculated in various subgroups such as Groups IIa and IIb or those children with and without certain cofactors (e.g., receiving drugs for treatment of HIV virus).

The primary hypotheses (H1 and H2) of comparing complication rates at specific time points in children with and without HIV-infection (Groups IIa and IIb) will be evaluated using the Mantel-Haenszel chi-square test stratifying on important potentially confounding variables such as IVDA of the mother. If there are several factors that need to be adjusted for simultaneously, a logistic regression analysis will be used to compare the rates with the factors as independent variables in the model along with an indicator variable representing group assignment. Similar analyses also apply to Hypotheses H3 and H4.

The persistence of cardiac abnormalities from the fetus to the neonate (Hypothesis H5) can be evaluated using McNemar's chi-square. If the abnormality is measured using a continuous variable (e.g., wall thickness from an echocardiogram), a paired t test or Wilcoxon signed rank test can be used.

To determine which cofactors are associated with cardiac or pulmonary abnormalities (Hypotheses H6 and H7), a logistic regression analysis will be done.

To determine whether diagnostic methods are able to detect cardiac or pulmonary disease before signs or symptoms appear in HIV-infected children (Objective O2), the sensitivity, specificity and positive and negative predictive values of each method will be determined. In order to determine the optimal cut-point for a diagnostic measure, a receiver operating characteristic (ROC) analysis will be performed (Metz, 1978). Combinations of diagnostic methods that may better detect disease will be evaluated using logistic regression analysis. The specific diagnostic methods likely to be of value are: LDH or PO_2 (or $A\text{-}aO_2$) for PCP, IgG for lymphoproliferative lung disease, echocardiogram measures (e.g., fractional shortening) for ventricular disease EKG measures (e.g., PR interval) for detecting arrhythmia, and DTPA for detecting early lung disease.

To evaluate whether PCP accelerates the rate of lung function decline, (Objective O4), the decline in children with and without PCP can be compared use an unpaired t test or a Wilcoxon rank sum test. More elaborate longitudinal data analyses are described in Section 7.3. To determine whether PCP shortens the life span of HIV-infected children with chronic lung disease (Objective O4), a survival analysis will be performed. Detailed discussion of such an analysis is described in Section 7.4.

All hypotheses and objectives referring to HIV-infected children apply to children in Groups I and IIa.

7.3 Longitudinal Analyses

Various clinical and laboratory measurements will be made periodically during the 2-1/2 to 4-1/2 year follow-up period. Most longitudinal methods typically require balanced data (i.e., no missing observations) which is unlikely to be true in this study. Alternatively, meaningful summaries of the longitudinal experience for each patient will be developed such as the rate of change from baseline, and these summary measures can be compared between groups using t tests or Wilcoxon rank sum tests.

Changes in continuous measurements (e.g., PaO_2) over time will be compared by calculating a slope over time for each patient and comparing mean slopes between groups. The validity of the linearity assumption will be investigated. Due to varying lengths of follow-up caused by staggered enrollment, termination of the study, dropouts, and mortality, there will be different numbers of measurements between subjects and inequalities in the precision of estimates of individual slopes, so a weighted mean slope will be used to determine group means and variances. These weighted mean slopes can be compared between groups using a weighted regression model which can also include covariates from baseline.

The missing data problem may require more sophisticated data analysis approaches. Fortunately, much recent work in the analysis of longitudinal data with missing values has been done. In October, 1986, the NHLBI held a workshop on "Methods for Long-term Data Analysis in Epidemiological and Clinical Studies." All the papers presented at this workshop have been published as Volume 7, 1988, Statistics in Medicine. In addition, a review of statistical methods for longitudinal data has been given by Cook and Ware (1983) in which analysis approaches for continuous and dichotomous data are discussed. Also, a discussion of linear model approaches for the analysis of longitudinal studies has been given by Ware (1985). A unified approach useful for modeling continuous or categorical longitudinal data has been developed by Liang and Zeger (1986) using a generalized estimating equations approach. In addition, Schluchter has developed methodology to analyze continuous repeated measures data when the covariance structure is specified (Jennrich and Schluchter, 1986; Schluchter, 1988). The disadvantage of all these methods is that missing observations are assumed to be missing at random, i.e., not related to the process being studied. For example, if mortality during the study is related to a subject's rate of decline of CD4/CD8 ratio, then the values of CD4/CD8 ratio that are missing because that subject died during the study are not missing at random. This type of nonrandom missingness has been termed "informative censoring" (Wu and Carroll 1988). At present, there are only a few models available to handle informatively censored longitudinal data. The Wu and Carroll (1988), method uses a random effects linear model where the missing process is modelled assuming a probit function that allows tractable estimation, Wu and Bailey (1988), consider simpler ad hoc noniterative techniques for handling informatively censored data, and Wu and Bailey (1989) propose a general linear models approach.

7.4 Analyses of Time-To-Event Data

Survival (time-to-event) analysis methods will be used to analyze morbidity and mortality outcomes and to identify prognostic factors for each event (e.g., HIV infection). Kaplan-Meier (Kaplan and Meier, 1958) estimates of "survival" curves will be obtained for each subgroup of interest. The construction of confidence interval estimates around a point of the survival curve will use the estimate of standard error suggested by Peto, Pike, et al (1977). Comparisons between subgroups (e.g., those children with and without confirmed HIV infection) will be made using the Mantel-Haenszel test (Mantel, 1966). These methods are useful in that they allow for varying length of follow-up and permit the comparison of the entire survival experience during the follow-up period. Kaplan-Meier estimates of time to determination of HIV infection will be developed for children in Group II.

To evaluate covariates that may relate to mortality and morbidity, the Mantel-Haenszel (log rank) test and Cox's (1972) lifetable regression model will be utilized. Graphical tests examining log(-log) plots of survival will be examined to check the assumption of proportional hazards, and stratified versions of these methods will be used when appropriate. Important baseline covariates to consider will include: immunological status (e.g., IgG, CD4/CD8 ratios) and baseline medical conditions present. Time-dependent covariates, including the most recent immunological and hemodynamic measurements as well as any drug treatment being given, will also be examined as prognostic factors in the Cox model. The use of log-linear models for survival data (Holford, 1980; Laird and Oliver, 1981), and the procedure proposed by Mantel and Byar (1974), and a cubic spline proportional hazards model using the new SAS PROC PHSPLM procedure will be explored as a less computationally intensive methods of dealing with time-varying covariates as compared to Cox regression. In the Study, basic admission and laboratory data should be available on all subjects. However, it can be expected that information on some baseline and time-varying covariates will be missing for individual subjects. For selected covariates that are partially observed, descriptive analyses will be performed comparing those subjects having the measurement with those subjects missing the measurement, in order to help determine if the covariate can be considered missing at random. The standard Cox regression and log-linear analyses of survival leave out subjects having missing data for any covariates included in the model. If these analyses lead to a substantial loss of subjects (say >10%), then a new technique for analyzing survival data with missing covariates using maximum likelihood via the EM algorithm (Schluchter and Jackson, 1989) will be used and results will be compared to the analysis of complete cases.

7.5 Summary

The analysis of data in this "natural" history study requires special care. In analyzing the data, careful attention will be given especially to the problem of bias that might arise from incomplete reporting.

8. Quality Control

Quality Control will be monitored by a committee comprised of one representative from each Clinical Center and one representative from the Clinical Coordinating Center and the NHLBI. Members of the Quality Control Committee will have diverse backgrounds (e.g., pulmonology, cardiology, infectious diseases, immunology, data management, biostatistics, etc.) to assure competence of the committee in diverse areas of their review. This committee will develop criteria for performance of the protocol and the Clinical Coordinating Center will provide individuals who will visit each Clinical Center annually to measure compliance with the developed criteria. The Quality Control committee will review the findings obtained at the site visits and advise centers of their performance and report findings to NHLBI. Although annually on site evaluations (audits) will occur; annually, semiannually, quarterly, and monthly evaluations will be done by the Clinical Coordinating Center from reported data. A checklist will be completed by each Clinical Center and forwarded to the Clinical Coordinating Center documenting equipment, supporting staff, and collaborative arrangements in place prior to the beginning of phase II.

8.1 Clinical Center Clinical and Laboratory Procedures

8.1.1 History and Physical

Compliance with the study protocol regarding cardiopulmonary history and physical exam will be determined by an on-site random review of subject records, log books and other records. At the Clinical Coordinating Center, a review of submitted data forms for completeness and accuracy will be ongoing. The following areas will be assessed.

1. Enrollment procedures;
2. Adherence to study protocol;
3. Adherence to the performance of the indicated diagnostic studies on subjects requiring symptom evaluation; and
4. Completeness of data forms.

History and physical examination variability are likely to be large. In this situation, gross classification of major diseases may be the only method of acceptable data reliability.

8.1.2 Clinical Immunologic/Virologic Procedures

Since all of the participating centers in the NHLBI contract are members of the AIDS Clinical Trials Group (ACTG), all centers have quality control procedures for HIV testing (culture, ELISA, Western Blots) and certain immunologic tests (leukocyte markers, CD4/CD8). The ACTG's Quality Control Laboratory for HIV testing is located in Dr. F. Blaine Hollinger's Virology Laboratory at Baylor College of Medicine. Dr. Hollinger supervises the periodic testing of HIV cultures performed at ACTG institutions, including the five associated with this contract.

Similarly this project can take advantage of a quality control mechanism already established for the five participating institutions. Through a contract to the NIAID, Fast Systems, Inc., (FSI) 211 Perry Parkway, Gaithersburg, MD 20877, (301)977-0536, is providing a Flow Cytometric Quality Control Program to the ACTG study sites. Unknowns are sent by FSI monthly and institutions report back leukocyte subset data for quality control analysis. Thus, CD4/CD8 data gathered for this study will benefit from the NIAID quality control program involving the five sites.

With this study's tests that are not quality controlled by the ACTG mechanism, internal quality control programs of each institution will be relied upon. For example, each institution performs CMV and EBV virus cultures or has these cultures done in a reference laboratory that meets institutional quality control criteria. Appropriate positive and negative control cultures are performed periodically to document accuracy of the laboratories. In like fashion, the five institutions exert quality control over the serological tests for CMV and EBV and for serum immunoglobulin quantitation. The Quality Control Committee of this project will meet to compare and standardize testing procedures and quality control checks of each of the five institutions. Each center will keep a log of all procedures which overlap with other ongoing protocols to avoid repetition.

8.2 Clinical Center Pulmonary Procedures

8.2.1 Chest X-rays

The chest films will be read on the basis of criteria established by the pediatric radiologic consultant panel prior to the beginning of the study. This scoring system for the diagnosis and staging of interstitial lung disease from routine chest x-rays, a modification of the technique of McCloud et al, 1983; has been the outgrowth of an ad hoc Taskforce on Pediatric Interstitial Lung Disease which met at the NHLBI during 1988-89. A review of a representative selection chest films will be performed in a blinded manner quarterly by a panel of radiologists from the participating centers, including the Coordinating Center. Films will be evaluated for technical quality as well as the accuracy of the interpretation. The consultant panel will be used to resolve discrepancies in readings from individual centers.

8.2.2 Oxygen Saturation and Arterial Blood Gas Analysis

In the measurement of arterial oxygen saturation, the pulse rate as determined by the saturation sensor attached to the patients finger or toe must be within 2-4 beats per minute of the heart rate measurement from impedance monitoring. Saturation must meet this criteria for at least 6 of the 10 minute observation period. Respiratory rate will be determined in parallel with this monitoring as a check on the adequacy of patient ventilation during the study. The patient must have been breathing quietly at rest without breathholding during the portion of the study from which the data is accepted. With assisted ventilation, there must be no interruption of ventilation during the test period.

Arterial oxygen tension measurement is dependent on acceptable blood drawing technique. The blood must flow passively into the syringe and there must be evidence of pulsatility. The blood gas analyzer will have a two point calibration within 15 minutes of the analysis. Hemoglobin will be used to correct the bicarbonate and base deficit values.

8.2.3 Pulmonary Function Tests

To insure consistent, reproducible results from pulmonary function testing in this study, all centers will use the same pulmonary function equipment. The pulmonary function data will be collected by a microcomputer. The same software for collection and calculation of the results will be employed at all centers participating in this study.

The adequacy of the quality of pulmonary physiology measurements will be determined by first comparing results of pulmonary function tests from control patients (e.g., those with no other evidence of lung disease) in the centers participating in this study to published controls and then comparing the results between the centers. These results will be compared on a monthly basis by a group appointed by the pulmonary subcommittee and a complete, in-depth analysis will be performed by the Coordinating Center twice a year during the study period. Both intrasubject and intersubject analysis will be performed. At each pulmonary function study at least three repetitions of each pulmonary function parameter will be obtained before the best effort is selected. However, all three efforts will be reported to the Coordinating Center. A center reporting data which varies greater or less than 10% from the mean for all centers will be required to study their pulmonary function testing procedures to assess for the nature of the discrepancy. They will also be asked to retrain their technical staff in the methodology for the test performance.

8.2.4 Aerosolized Tc-99m DTPA Scintigraphy (DTPA studies were discontinued on February 10, 1993 on the recommendation of the Steering Committee)

To assure uniformity of technique and results in the DTPA aerosol studies, quality control records will be kept in log books at each site and, on interchangeable PC discs. Records will be kept in four major quality control areas.

1. Tc99m-DTPA radiochemical quality testing: Using 100% acetone solvent and simple paper strip chromatography, the percent free TcO4 in each DTPA preparation will be determined prior to each study and afterward on any residual nebulizer fluid for each study. Using chromatography results as a quantitative basis for free Tc, clearance results will be corrected for any rapidly clearing Tc that might have been present. Corrected and uncorrected DTPA clearance rates will be recorded.

2. Gamma camera performance will be documented by including photos of a Tc-99m flood field from a point source obtained from the camera the day of the study and by recording the times to collect the original and uniformity-corrected fields.

3. Camera-computer quantitative performance will be assessed by obtaining a 3 minute digital flood field using the point source method. This flood will be compared visually with the analog flood to look for computer-introduced areas of inhomogeneity. A second 3 minute digital flood also will be collected in twelve increments of 15 seconds each. These floods will be compared visually with the other floods and the total counts from the incremental collection will be added to determine if they match the total counts collected over the single 3 minute collection period.

4. Data analysis - clearance rates will be determined in percent/min and as half-time of clearance. Any changes in count rate during the clearance study greater than 3 SD will require that the regions of interest (ROI) and patient's position for the unusual frames be reviewed. If motion is detected, the ROIs will be repeated by hand for each frame. A new time-activity curve and a new clearance rate will then be generated from the corrected ROI.

8.2.5 Bronchoalveolar Lavage

Each center will perform safe bronchoscopy as per the Manual of Operations. Possible complications will be minimized by careful observation and intervention as detailed here and in the manual of operations.

Test time — preparation and sedation - 60 minutes;
procedure - 15-30 minutes;
total: 1 1/2 hours

Equipment

The pediatric bronchoscope will be maintained appropriate to the manufacturers specifications. Cold or gas sterilization will be completed with vigorous cleaning following each procedure. The light source will be maintained per manufacturer's specifications and a spare light bulb available on the bronchoscopy cart. Video or still photography will document structural abnormalities. Cardiopulmonary monitors and oximeters will be maintained and calibrated per manufacturer's specifications.

Each procedure will occur in the appropriate location determined by the individual clinical centers with adequate personnel to monitor the patient following informed consent. Informed consent will be obtained. Patients old enough to comprehend will be addressed directly to familiarize them with the procedure to improve compliance and minimize fear.

8.2.6 Pathology Studies

Review of diagnostic biopsies, autopsies, and BAL fluid for consistency of diagnosis will be by a panel of three pathologists from the participating

institutions. When there is disagreement a consensus diagnosis will be made and data accordingly revised. Standardized methods with appropriate controls will be employed by all centers.

8.3 Clinical Center Cardiac Procedures

8.3.1 Electrocardiogram and Holter

Electrocardiogram

Electrocardiograms (ECG) will be measured at each center using methods outlined in the manual of operations of this protocol. Interpretation will be standardized depending upon the measurements (e.g., if R wave amplitude in lead V^6 exceeds the Davignon criteria for age, left ventricular hypertrophy will be diagnosed). During the yearly audit of each center, 10% of the ECG's will be examined to be sure that the tracing is free of motion artifact for at least 10 seconds and that the measurements of the amplitude agree within 100 microvolts.

Holter

Holter reports will be reviewed in each center. Any abnormalities in the numeric report (e.g., number of premature beats) must be reviewed and the number of beats verified by a physician. The high heart rate, low heart rate and any abnormalities of conduction of rhythm must be documented with electrocardiographic tracings. Any Holter report with a disturbance of conduction or rhythm (other than sinus tachycardia or sinus bradycardia) will be sent to the coordinating center for distribution to the ECG/Holter review center at Houston.

8.3.2 Echocardiogram

8.3.2.1 General Principles; Personnel

An experienced physician echocardiographer and Pediatric Echocardiography technologist will be designated at each Clinical Center and whenever possible will be present for studies on protocol patients to ensure that appropriate and medically acceptable patient care procedures and laboratory safety principles are followed.

Each center will record and document prenatal and postnatal echocardiograms in accordance with its own protocols for such studies. Current individuals designated to monitor quality assurance will continue at their own institutions, to accomplish the following:

1. Ensure examinations are performed in a complete and uniform manner;
2. Facilitate the acquisition and recording of data needed for this particular contract protocol and obtained from enrolled patients; and

3. Provide ongoing "on-site" training for all local personnel involved in the performance of these studies to assure inter-observer reproducibility (especially in view of the relatively long course of this protocol).

8.3.2.2 Standardization; Date Acquisition and Analysis

The five Clinical Centers involved in this protocol have well-established laboratories with demonstrated proficiency in fetal and pediatric echocardiography. 2-D imaging and M-mode analysis are now standardized by the use of generally agreed upon view (or planes) and measurement techniques described by the American Society of Echocardiography (Henry et al, 1980). Methods to assess and quantitate pressure gradients by Doppler echo reliably are now agreed upon (Hatle and Angelsen, 1985), and semi-quantitative measure of valvar regurgitation accepted (Hatle and Angelsen, 1985); both require view standardization. The above will be used to asses anatomy and function in study patients, and data necessary for the protocol will be gathered by the investigators from each laboratory's routine echocardiography techniques. Measurement of chamber sizes, transvalvar flow, and umbilical artery flow in the fetus as well as right ventricular size and transvalvar flow in postpartum studies will be done at respective clinical centers.

Newer methodology to assess left ventricular performance, loading condition, and contractility (Section 5.3.3) has been published and its clinical efficacy demonstrated (Lipshultz et al, 1989); measurements required for determination of these parameters will be performed at all Clinical Centers after an appropriate familiarization and standardization period. This will be achieved by having personnel from Boston Children's Hospital travel to and work with responsible personnel at each of the centers to establish uniform methods of data acquisition. In addition, video recorded data will allow review of m-mode methods, permitting the center performing data analysis to recognize variability in data recording techniques.

Standardization of echocardiographic measurements will be performed by centralization of data analysis. Classical M-Mode, 2-D and Doppler echocardiographic indices of left ventricular size, wall thickness, shortening fraction, and transvalvar flow in the fetus and extrauterine patients are readily standardized measurements. However, in a multi-center study, analysis of wall stress is confounded by inter-institutional differences in measurement devices and interobserver variability. Quantitation of wall stress and related measures in this protocol will therefore be centralized to a single clinical center. The use of a designated technologist and an automated wall stress analyzation program minimizes interobserver variability and contains equipment costs. The individual performing the data analysis will be blinded to clinical status, thereby facilitating an unbiased determination of echocardiographic abnormalities.

8.3.2.3 Equipment

Equipment will be standard at all clinical centers to conform with the manual of operations.

8.3.2.4 Fetal and Postnatal Echocardiographic Evaluations

The Clinical Centers will use a standardized work sheet for data collection and transmission. In addition to information specific to the cardiovascular system, the fetal studies will identify information about fetal maturity, non-cardiac abnormalities and hydrops fetalis. Standardized fetal (See Section 5.3.1) and pediatric echocardiographic views (see above) will be used by all centers to assess cardiac anatomy and function. Postnatal data related to wall stress analysis and mass will be transmitted (via videotape or hard copy) as soon as possible to Boston Children's Hospital for analysis. Centralization of data analysis will help reduce interobserver variation. All data will be preserved in duplicate, with each center making calculations from original recordings. All data, both local and centralized, will be forwarded to the Clinical Coordinating Center for analysis and quality control.

9. Infection Control Procedures

All five participating institutions in this project will conduct research in accord with the universal blood precautions of the Centers for Disease Control (See Appendix). Each institution will have in place a Policies and Procedures Manual for dealing with employee exposure to HIV (See Appendix). Each institution will meet all requirements for protection of healthcare workers as prescribed by the Occupational Safety and Health Branch, NIH Division of Safety and CDC (See Appendix).

10. Standards for Patient Care

Patient Counseling Procedures will be in follow the recommendations expressed in the "Report of the Presidential Commission on the Human Immunodeficiency Virus Epidemic", June 1988. Chapter VI, specifically addresses issues of testing and counseling. Counseling procedures sill be consistent with the guidelines established for "The Women and Infant Transmission Study" (WITS). Proposed procedures and "HIV Education and Counseling Checklist" from the WITS protocol are included. The NIMH published "Coping with AIDS" and "Questions and Answers about HTLV-III Antibody Test" will be made available to all health care professionals involved in the study.

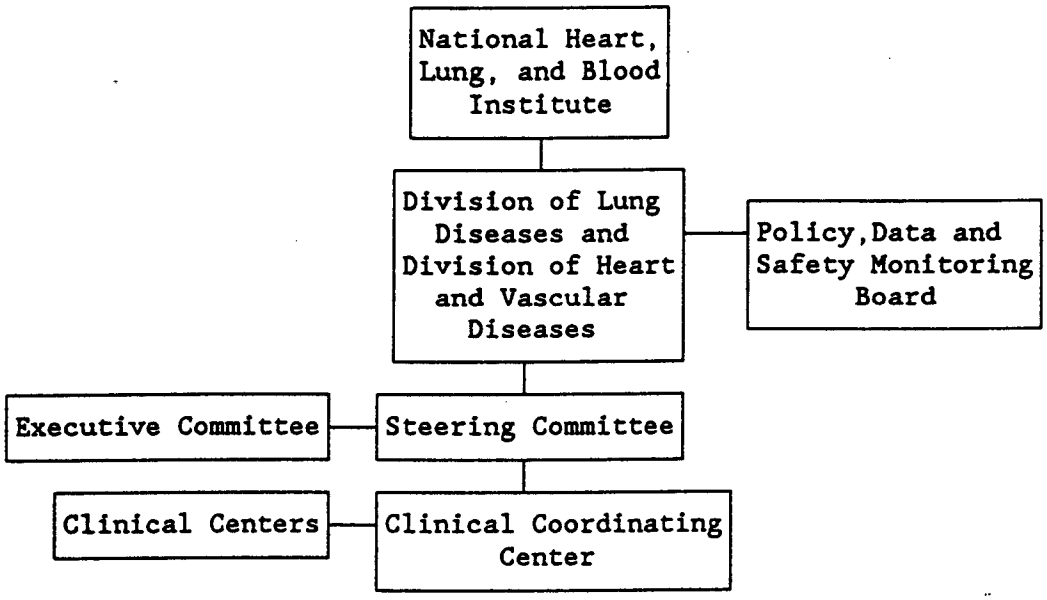
11. Organizational Structure

11.1 Introduction

The organizational components of this study include participating units and administrative units. The participating units are: individual Clinical centers, a Clinical Coordinating Center, and the National Heart, Lung, and Blood Institute. The administrative units are: a Policy, Data and Safety Monitoring Board, a Steering Committee, an Executive Committee and several working subcommittees established by the Steering Committee.

Figure 7

Organizational Chart



11.2 Participating Units

The duties and responsibilities of the participating units in this study are described below.

11.2.1 Clinical Centers

Each of the Clinical Centers is responsible for recruiting the required number of patients, administering the clinical evaluation and diagnostic tests as required by the study protocol, obtaining follow-up information, and collecting, recording, and forwarding patient data to the Clinical Coordinating Center.

The professional and clerical organization of each Clinical Center will include a senior clinical investigator in pediatric cardiology and pediatric pulmonology, a senior investigator or consultant in immunology/infectious diseases and associate investigators and consultants, both M.D.'s and Ph.D's, in a variety of other specialties such as obstetrics, pathology, nuclear medicine, radiology, a nurse coordinator, a patient interviewer, a nurse/social worker, data entry personnel, and laboratory and clerical support staff. The Principal Investigator will be a member of the Steering Committee and will be responsible for the ongoing operation of the study. The clinical nurse coordinator will be responsible for obtaining follow-up data, checking the completeness of data and forwarding it to the Clinical Coordinating Center, and shipping any studies or specimens to central locations as needed for interpretation, quality control or storage. Clinical Center staff will meet twice a month to review local study progress, report on work completed and bring up operational problems, some of which may be brought to study-wide attention by the Clinical Center's Principal Investigator. The Principal Investigators have a forum for considering study-wide problems in the Steering Committee and via conference calls.

The Institutions participating in the study as Clinical Centers and their respective Principal Investigators are listed below.

1. Baylor College of Medicine
   Houston, Texas
   Dr. William Shearer, Principal Investigator

2. The Children's Hospital/Harvard Medical School,
   Boston, Massachusetts
   Dr. Steven Lipshultz, Principal Investigator

3. Mount Sinai School of Medicine
   New York, New York
   Dr. Meyer Kattan, Principal Investigator

4. Presbyterian Hospital/Columbia University
   New York, New York
   Dr. Robert Mellins, Principal Investigator (May, 1991 - present)
   Dr. Frederick Z. Bierman, Principal Investigator (1990 - May, 1991)

5. UCLA School of Medicine
   Los Angeles, California
   Dr. Samuel Kaplan, Principal Investigator

Among the specific functions of the Clinical Centers are:

1. Providing representation at each Steering Committee meeting;

2. Participating in the development of the protocol, Manual of Operations and data collection forms;

3. Recruitment of infants and children who meet the eligibility requirements;

4. Performing the specified laboratory, pulmonary and cardiac tests;

5. Follow-up of patients;

6. Transmitting specified data to the Clinical Coordinating Center; and

7. Producing reports as required.

11.2.2 Clinical Coordinating Center

The Clinical Coordinating Center plays a major role in the design, implementation, and execution of the study. The Clinical Coordinating Center will be represented on, and will work under the direction of the Steering Committee. Staff of the Clinical Coordinating Center has the responsibility of collecting, editing, storing, and analyzing all data received from the Clinical Centers and the External Review Consultants.

The Clinical Coordinating Center for this study is the Cleveland Clinic Foundation, Cleveland, Ohio. Mark Schluchter, Ph.D., is the Principal Investigator.

The CCC responsibilities will include:

1. Organizing Steering Committee meetings and providing the minutes for each;

2. Providing leadership for protocol design and development;

3. Participating in the development of the Manual of Operations;

4. Coordinating training for personnel from the Clinical Centers both in the areas of Data Management and the areas of collecting pulmonary and cardiac data to assure that uniform techniques are used throughout the study;

5. Configuring all equipment at the Clinical Centers with software in the CCC and delivering it "turn key" ready to the Centers;

6. Developing study forms and pretesting the procedures for data recording, processing and reporting;

7. Collecting data from the Clinical Centers and reviewing and editing all data transmitted to the Clinical Coordinating Center;

8. Checking the completeness of records and periodically preparing performance reports to participating Clinical Centers;

9. Analyzing periodically the frequency of adverse side effects of the diagnostic procedures and reporting this data to the Policy, Data and Safety Monitoring Board;

10. Participating in the establishment and monitoring of quality control procedures and providing quality control of data received;

11. Monitoring patient recruitment at each Clinical Center;

12. Storage of data in a computerized format suitable for statistical analyses;

13. Providing comprehensive annual and brief monthly reports on the status of the study to the Clinical Centers and the NHLBI;

14. Providing quarterly financial reports to the NHLBI; and

15. Providing analyses needed for writing the results of the study.

Clinical Coordinating Center staff meet weekly to review study progress, report on assigned work and receive work assignments to fulfill the Clinical Coordinating Center specific functions.

11.2.3 National Heart, Lung, and Blood Institute Project Office

The Division of Lung Diseases (DLD) and Division of Heart and Vascular Diseases (DHVD), National Heart, Lung, and Blood Institute, as sponsors of the study, are responsible for providing organizational, scientific, and statistical direction to the study through the Airways Diseases Branch, DLD. The Scientific Project Officer, Dr. Hannah H. Peavy, is a voting member of the Steering Committee and a non-voting member of the Policy, Data and Safety Monitoring Board. The Contract Officer, Mr. Douglas Frye, is responsible for all administrative and fiscal matters related to the award and conduct of the contracts.

11.3 Administrative Units

The participating units of the study are coordinated by the DLD, the Policy, Data and Safety Monitoring Board, and the Steering Committee.

11.3.1 Policy, Data and Safety Monitoring Board

The Policy, Data and Safety Monitoring Board acts in a senior advisory capacity to the NHLBI on policy matters throughout the duration of the study. In addition, it periodically reviews study results and evaluates the study diagnostic procedures for beneficial and adverse effects.

The Board is composed of a chairman and additional voting members, who are appointed by the NHLBI for the duration of the study. The Scientific Project Officer, as an ex-officio member, is a non-voting member of the Board. Board meetings are attended, when appropriate, by senior representatives from the Clinical Coordinating Center and the chairman and subcommittee chairmen of the Steering Committee. Additional Board members or consultants may be appointed,

if deemed necessary, by the NHLBI in response to recommendations by the Board. No voting member of the Policy, Data and Safety Monitoring Board may participate in the study as an investigator; however, other investigators from the Board member's institution will not be excluded from participating in the study. The Board will meet no less than twice a year.

Specific functions of the Policy, Data and Safety Monitoring Board are:

1. To review and approve the study protocol and Manual of Operations;

2. To review and analyze the progress of the study, including the clinical data to evaluate its relevance to the program goals;

3. To monitor the study diagnostic procedures for beneficial and adverse effects on the patient;

4. To approve major changes in the protocol or Manual of Operations and make recommendations to the NHLBI;

5. To review and approve ancillary studies (with the possible effect on the main study being the major criterion);

6. To assist the NHLBI in resolutions of problems referred by the Steering Committee;

7. To make recommendations to the NHLBI on any proposed early termination of the study because of adverse effects of any diagnostic procedure; and

8. To recommend remedial measures or discontinuation of individual Clinical Centers which perform unsatisfactorily.

11.3.2 Steering Committee

The officers of the Steering Committee are a Chairman (Robert Mellins M.D.), Chairmen of the three major subcommittees Pulmonary (Arnold Platzker, M.D.), Cardiology (Samuel Kaplan, M.D.) and Study Population/Immunology (William Shearer, M.D.), and a Recording Secretary. The Chairman and Subcommittee Chairmen will be of different professional disciplines and from different participating hospitals. A member of the staff of the Clinical Coordinating Center will serve as the Recording Secretary. The Chairman, in consultation with the Project Officer and the Clinical Coordinating Center, will determine the agenda for the meeting. The Chairman will also preside over all the Steering Committee meetings and appoint members to ad hoc subcommittees. One of the Subcommittee Chairmen shall serve in the absence of the Chairman. If, for any reason, the Chairman is unable to complete his term in office, the DLD will appoint a new Chairman. The Recording Secretary will record and distribute minutes of Committee meetings, notify members of meetings, keep and distribute protocols and other Committee documents, and maintain files of all Committee

activities including files of scientific data.

The Steering Committee, composed of representatives from the Clinical Centers, the Clinical Coordinating Center and NHLBI, provides scientific direction to the study at the operational level. The voting members of the Steering Committee are one member from each Clinical Center and the Clinical Coordinating Center, and the DLD Project Officer. The NHLBI appoints the Chairman of the Steering Committee and the Subcommittee Chairman. The Chairman of the Steering Committee provides leadership for the Committee in performing its tasks, chairs the Steering Committee, makes presentations to the Policy, Data and Safety Monitoring Board, and acts as a liaison to the NIH advisory groups. The Steering Committee meets six times in the first year and three times a year thereafter. Voting is done by majority rule with each Center and the Project Officer having one vote.

Specific functions of the Steering Committee are:

1. To design the protocol and patient forms and review the Manual of Operations;

2. To make recommendations to the Policy, Data and Safety Monitoring Board concerning changes in the protocol and Manual of Operations;

3. To review and analyze the progress of the program;

4. To review all proposed ancillary studies and to report all recommendations to the Policy, Data and Safety Monitoring Board (the major criterion being the possible effect on accomplishing the objectives of the main study);

5. To monitor the performance of the individual Clinical Centers with regard to patient recruitment and patient follow-up studies; and

6. To be responsible for the presentation of the program results to the biomedical community.

The Steering Committee will meet no less than twice a year. Five members of the Steering Committee constitute a quorum. The deliberations of the Steering Committee will be conducted in a parliamentary manner. Unless otherwise specified all decisions will be taken based on a simple majority of those present and voting, providing a quorum is established. The Steering Committee may act in meetings, by mail or by telephone, with written confirmation to the Chairman if voting is done by telephone. Special meetings of the Steering Committee shall be called by the Chairman, at the request of NHLBI, or at the written request of a majority of the members of the Steering Committee.

11.3.2.1 Consultants

The Steering Committee, with the concurrence of NHLBI, may invite as consultants individuals whom it feels would contribute useful information to the Steering Committee deliberations.

11.3.2.2 Voting

Each member has one vote concerning amendments to the protocol or on any other matters brought before the Steering Committee for a vote. Each individual appointed to a subcommittee, including consultants, will have one vote in subcommittee meetings.

11.3.2.3 Subcommittees

The Steering Committee may establish or abolish any subcommittee it determines to be in the study's best interest. The membership of any subcommittee will be determined by the Chairman with the approval of the Steering Committee. No subcommittee may present a report outside the Steering Committee unless it has been specifically authorized to do so by the Steering Committee.

11.3.2.4 Publications and Presentations Subcommittee

This subcommittee will review all written and oral presentations on the design, progress and results of the study, including any ancillary studies. The subcommittee will follow National Heart, Lung, and Blood Institute guidelines on presentations and publications.

11.3.3 Executive Committee

The Executive Committee is constituted of the Chairman, Chairmen of the three major subcommittees, (Pulmonary, Cardiology, and Study Population/Immunology) the Director of the Clinical Coordinating Center, and the DLD Project Officer. The Executive Committee is the governing body of the Steering Committee. It shall have general supervision of the affairs of the Steering Committee between meetings. The Executive Committee is subject to the order of the Steering Committee and none of its actions shall conflict with the actions taken by the Steering Committee.

The Executive Committee will meet or hold telephone conferences between Steering Committee meetings to review interdisciplinary issues on the Steering Committee agenda.

Specific functions of the Executive Committee are:

1. To make recommendations to the Steering Committee concerning interdisciplinary issues;

2. To assign to subcommittees responsibility for detailed review of source information on issues with interdisciplinary implications;

3. To review cardiology, pulmonary and infectious disease/immunology procedures for consistency with each other;

4. To review data collection and interpretation issues for compatibility across the study disciplines; and

5. To promote communication within centers through feedback on interdisciplinary issues to the subcommittees.

12. Policy Matters

12.1 Adherence to Protocol and Minimum Patient Load

The ultimate success of the trial will depend upon adherence to the protocol and Manual of Operations and the admission of sufficient numbers of patients per center to the study (See Section 4.2). Failure to adhere to the protocol, Manual of Operations, or the patient recruiting requirements will be reviewed by the Project Officer and the Policy, Data and Safety Monitoring Board. Major infractions or suboptimal performance will result in termination of contract support.

12.2 Eligibility and Inclusion of Patients

It is of utmost importance that as little bias as possible be introduced into the selection of patients for inclusion in the trial. Therefore, patients with the criteria for inclusion (with no contraindications) who come to the attention of participating investigators, should be considered for admission to the study unless there is a lack of informed consent. The Principal Investigator is ultimately responsible for the necessary scheduling and coordination required for the follow-up examinations. If the patient dies during the follow-up period, the Principal Investigator will be expected to contact the patient's physician to obtain sufficient information to complete the data requirements and/or postmortem protocol.

12.3 Informed Consent

The policy of the Department of Health and Human Services stipulates that trials which involve human subjects must be preceded by assurance that the individual's safety, health, and welfare (including the rights of privacy) must not be infringed. Participation must be voluntary and the direct or potential benefits of the research must outweigh the inherent risks to the individual. Informed consent is difficult to define. Under the Department of Health and Human Services policy, the local institutions have the responsibility for protection of human rights with the guidelines provided by the Department.

A copy of the assurance of institutional compliance with this policy is required by the Project Office prior to the initiation of the study. This policy specifies that an informed consent must be obtained from all patients prior to entry into the trial.

Since it is recognized that this informed consent could introduce a bias into the study, considerable responsibility must rest with the physician seeking this consent. It has been suggested that informed consent may be an "uneducated" consent. It may be possible, after an explanation with no coercion, to obtain a signature on a document that would satisfy the Institutional Review Board. The reality of the situation, however, is that it is the rare subject who appreciates all the ramifications of his entry into a study and the inconveniences and risks involved. In fact, some of these risks may be truthfully unknown to the investigators. On the other hand, there is evidence to suggest that a too

detailed exposition of all the pros and cons of the study design and the possible side effects can confuse the average subject to the extent that, in essence, the physician ends up making the decision for the subject. Hopefully, both extremes will be avoided in this study and consent will be both informed and as educated as possible.

It is impossible to provide a single statement that can be used by all physicians in all situations with all patients in this study. The form to be used by each institution must satisfy the local Institutional Review Board. However, the consent form used by each institution must include as a minimum the information contained in the consent form found in Appendix 7.

For all clinically indicated procedures, for which a specific informed consent is required, consent forms already in use at each institution will be used.

12.4 Reports

The Clinical Coordinating Center will periodically distribute formal reports to NHLBI and the Clinical Centers as per contract agreement. A final report will be prepared including a complete description of all study activities and an in-depth analysis of all data. Such an in-depth statistical analysis would include characterization of the study population, and morbidity and mortality of the study population.

12.5 Quality Control

Rigorous control for the data collection and recording will be maintained by the Principal Investigator at each Center as outlined in Chapter 8. The Principal Investigator or his designee at each Center will have the responsibility of scrutinizing the data and giving final approval before it is forwarded to the Clinical Coordinating Center.

The Steering Committee and NHLBI will develop methods and schedules to assess and evaluate the accuracy of the data being collected in each Clinical Center to ensure an adequate level of data quality throughout the Centers. The Principal Investigator agrees to take whatever action necessary to maintain the accuracy and quality control determined by the Committee and DLD. To the extent possible, the Clinical Coordinating Center will review all data submitted to the Center to ensure that it is free from errors and inconsistencies.

12.6 Ancillary Studies

Ancillary research studies may be conducted by the Clinical Centers if approved by the Steering Committee, Program Office, and the Policy, Data and Safety Monitoring Board. These research studies are considered to be a resource for the total program. Individual investigators will have the opportunity, however, to publish the results of their ancillary research activities separately.

Ancillary studies involving patients can in no way interfere with the patient care prior to patient assignment or the subsequent diagnostic regimen. The purpose of this interdiction is to assure a homogeneous application of the study protocol to all patients. Possible ancillary studies are discussed in Section 13.

12.7 Veto

The Director, Division of Lung Diseases, NHLBI, is empowered to exercise a veto on any decision of the Steering Committee which he/she considers not in the interest of the study. The veto, if exercised, should be communicated in writing to the Chairman within 30 days of the Steering Committee decisions.

13. Publications

A more detailed review of the policy and administrative guidelines for publication and presentation is contained in the Manual of Operations, Chapter 6.

13.1 Reporting Methods

The following define the means by which this study will be publicized and/or results reported.

1. Press Release: A document given to radio, television, newspapers, journals and periodicals.

2. Interview: Any discussion with a member of the press, science writer or radio or TV commentator who provides information for public dissemination.

3. Presentation: The delivery of information to scientific, professional or public groups that may be disseminated as a press release. A seminar within a closed academic setting is not classified as a presentation.

4. Publication: Any document submitted to a professional journal, book or any periodical with national circulation.

13.2 Goals

The following are goals the Steering Committee wishes to achieve, through their reporting mechanisms, as defined in Section 13.1, for press releases, interviews, presentations, and publications.

1. To disseminate the maximum amount of information, by publication, pertinent to the outcome of the study;

2. To share the results pertaining to ancillary studies;

3. To make the first priority of publication those related to major results;

4. To present the highest quality of publication possible;

5. To bring results of the study to publication in a timely manner with the participation and critique of all the investigators;

6. To be cognizant of all other studies involving the collaborative trial patients and potential publications on studies involving these patients; and

7. To make every effort to facilitate the publication and presentation of ancillary studies in a fashion that will not intrude upon nor have a deleterious effect on the presentation or publication of data from the overall study.

13.3 Content

Guidelines for each of the reporting mechanisms are governed in a similar fashion by the principles set by the NHLBI and the Steering Committee. All project-wide press releases, interviews, presentations, and publications must limit their substantive content to information items that have been described in the most recent request for proposals.

13.4 Review

The Publications/Presentations Subcommittee (PPS) will review draft publications with the following objectives in mind:

1. to make sure that no publication will have a deleterious effect on the study process, acceptance, or on the interpretation of its results;

2. to correct factual and conceptual inaccuracies;

3. to safeguard the rights of volunteer participants;

4. to prepare comments to assist collaborating scientists to publish papers of the highest quality (the latter is accepted as a responsibility because all publications related to this study will affect public perception of its scientific rigor and operational activities); and

5. to inform the Steering Committee and Policy, Data and Safety Monitoring Board of all public dissemination of information.

13.5 Distribution

After approval, a copy of all information being disseminated is sent to the NHLBI Project Officer, prior to each release, for approval and updating the program office files. A copy of the information is retained in a central file by the Clinical Coordinating Center, the Project Officer and the chairman of the Steering Committee.

13.6 Authorship

All official publications of the study will be written by a committee and credit for authorship will be to the "Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted Human Immunodeficiency Virus (HIV) Infection Study Group." Publication of results of ancillary studies performed on participants admitted to the study will be allowed by small groups or individual investigators. Approval by the PPS is required if the ancillary study makes use of data collected according to the study protocol. After the final results of the study are compiled and submitted for publication, individual investigators involved in the study may request access and publisher rights to data accumulated during the study.

Papers of the study which draw on data collected by all Centers will be identified by the PPS, with input from any of the participating investigators. Once a paper has been identified and approved by the PPS, the Steering Committee and all principal investigators will be made aware of the paper, and volunteers will be solicited from all participating investigators. The PPS, with the approval of the Steering Committee, may designate and appoint a chairman and members to a writing subcommittee for any study report. Each ad hoc committee will be charged with the responsibility for writing the paper in a prescribed time frame. The writing committee shall be automatically discharged when it submits its final report.

A detailed description of the authorship policy for various types of study manuscripts, is provided in Chapter 6 of the Manual of Operations.

Authorship of the paper will be clearly denoted as the "Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted HIV-Infection Study Group," with an asterisk to refer to the name of the Centers and their Principal Investigators. Under this title and the appropriate reference to the study's investigators as the main author of the paper, the names of the members of the ad hoc committee who prepared the paper will be printed in sequence. The first author listed will be the chairman of the writing committee or the person who undertook most of the work in the preparation of the paper, with other members listed in alphabetical order.

The authorship policy is intended to permit: (a) the investigators of all centers to be recognized and list the paper in their bibliographies; (b) the preparation of a paper by a small group of investigators; and (c) all investigators in all centers to have an opportunity to participate in the preparation of basic papers and gain academic recognition for their contribution to the program.

13.7 Publication of Endpoint Data

Endpoint data analysis may only be published prior to the completion of the study with the approval of the PDSMB. Publication of other data prior to the completion of the study should be encouraged by the PPS and Steering Committee providing the analyses do not contain endpoint data. Having stringent criteria for publication of endpoint data is extremely important since conclusions could easily be made erroneously. Research other than endpoint studies may be conducted at any time and is encouraged so that P^2C^2 may gain greater visibility in the scientific community by contribution of high-quality research.

14. Potential Ancillary Studies

Ancillary studies are projects which may contribute new, important information to the understanding of the pulmonary and/or cardiac consequences of vertically transmitted HIV infection, but have not been included in the protocol due to:

1. Insufficient funding to perform the study
2. Inability to perform the study at each of the funded clinical centers
3. Preliminary data or development of new technology required prior to initiation of the study

The following studies are presently being considered by the Steering Committee for designation as approved ancillary studies.

1. The relationship of P24 Antigen to the course of cardiovascular and pulmonary complications.
2. Serum catecholamines and ventilatory responses during sleep, to hypoxia and or hypercapnea in infants and children with vertically transmitted HIV infection.
3. Thin section CT scanning as an early predictor of lymphoproliferative lung disease.
4. DTPA clearance as a sensitive method to assess treatment of pulmonary complications
5. Heart rate spectral analysis as a probe of cardiac autonomic control in pediatric HIV infections
6. Identification of immune mediated primary cardiac and pulmonary injury.
7. Cardiac and pulmonary morphometry in patients following vertical transmission of HIV
8. Monoclonal antibody staining and PCR in early detection of cardiac and pulmonary viral infection
9. Relation of nutritional status to cardiovascular and pulmonary complications in HIV infection
10. Analysis of contractile protein isoforms in children with HIV infection and cardiac dysfunction

References

Abramowicz JS, Warsof SL, Arrington J, Levy DL, (1989). Doppler analysis of the umbilical artery: The importance of choosing the placental end of the cord. J Ultrasound Med, 8:219-221.

Acierno L, (1989). Cardiac complications in acquired immunodeficiency syndrome (AIDS): A review. J Am Coll Cardiol, 13:1144-1154.

Adler SM, Wohl MEB, (1978). Flow volume relationship at low lung volumes in healthy term newborns. Pediatr, 61:636.

Alderson PO, Biello DR, Gottschalk A, et al (1984). Comparison of Tc-99m DTPA aerosol and radioactive gases as adjuncts to perfusion scintigraphy in patients with suspected pulmonary embolism. Radiology, 153:515-521.

Alderson PO, Secker-Walker RH, Strominger DB, Markham J, Hill RL, (1974). Pulmonary deposition of aerosols in children with cystic fibrosis. J Pediatr, 4:479-484.

Allan LD, Crawford DC, Anderson RH, Tynan M, (1985). Spectrum of congenital heart disease detected echocardiographically in prenatal life. Br Heart J, 54:523-526.

Anderson DC, Hughes WT, (1987). Pneumocystis carinii pneumonia, in Feigin RD and Cherry JD (eds): Textbook of Pediatric Infectious Diseases (ed 2). Philadelphia: W.B. Saunders Company, pp 319-327.

Anderson DW, Virmani R, Reilly JM, et al (1988). Prevalent myocarditis at necropsy in the acquired immunodeficiency syndrome. J Am Coll Cardiol, 11:792-799.

Andiman WA, Martin K, Rubinstein A, et al (1985). Opportunistic lymphoproliferations associated with Epstein-Barr viral DNA in infants and children with AIDS. Lancet, 2:1390-1393.

Auger I, Thomas P, De Gruttola V, et al (1988). Incubation periods for pediatric AIDS patients. Nature, 336:575-577.

Bach FH, Sachs DH, (1987). Current concepts: transplantation immunology. N Engl J Med, 317:489-492.

Baroldi G, Corallo S, Moroni M, et al (1988). Focal lymphocytic mycocarditis in acquired immunodeficiency syndrome (AIDS): A correlative morphologic and clinical study in 26 consecutive fatal cases. J Am Coll Cardiol, 12:463-469.

Belman AL, Diamond G, Dickson D, et al (1988). Pediatric acquired immunodeficiency syndrome: Neurologic syndromes. Am J Dis Child, 142:29-35.

Bernstein LJ, Ochs HD, Wedgwood RJ, et al (1985). Defective humoral immunity in pediatric acquired immune deficiency syndrome. J Pediatr, 107:352-357.

Bharati S, Joshi VV, Connor EM, Oleske JM, Lev M (1989). Conduction system in children with acquired immunodeficiency syndrome. Chest, 96:406-413.

Birx DL, Redfield RR, Tosato G, (1986). Defective regulation of Epstein-Barr virus infection in patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related disorders. N Engl J Med, 314:874-879.

Blanche S, Le Deist F, Fischer A, et al (1986). Longitudinal study of 18 children with perinatal LAV/HTLVIII infection: Attempt at prognostic evaluation. J Peds, 109:965.

Blanche S, Rouzioux C, Guihard Moscato ML, et al (1989). A prospective study of infants born to women seropositive for human immunodeficiency virus type 1. N Engl J Med, 320:1643-1648.

Borow KM, Colan SD, Neumann A, (1985). Altered left ventricular mechanics in patients with valvular aortic stenosis and coarctation of the aorta: effects on systolic performance and late outcome. Circulation, 72:515.

Brenner M, Ognibene FP, Lack EE, et al (1987). Prognostic factors and life expectancy of patients with acquired immunodeficiency syndrome and Pneumocystis carinii pneumonia. Am Rev Resp Dis, 136:1199-1206.

Buck BE, Scott GB, Valdes-Dapena M, et al (1983). Kaposi sarcoma in two infants with acquired immune deficiency syndrome. J Pediatr, 103(6):911-913.

Bye MR, Bernstein L, Shah K, Ellaurie M, Rubinstein A, (1987). Diagnostic bronchoalveolar lavage in children with AIDS. Pediatr Pulmonol, 3:425-428.

Bye MR, Palomba A, Bernstein L, Shah K, (1987). Clinical Candida supraglottitis in an infant with AIDS-related complex. Pediatr Pulmonol, 3:280-281.

Campbell S, Bewley S, Cohen-Overbeek T, (1987). Investigation of the uteroplacental circulation by Doppler ultrasound. Semin Perinatol, 11:362-368.

Casagrande JT, Pike MC, Smith, (1978). An Improved Approximate for Calculating Sample Sizes for Comparing two Binomial Distributions. Biometrics, 34:483-486.

Centers for Disease Control (1985). Revision of the case definition of acquired immunodeficiency syndrome for national reporting-United States. MMWR, 34:373-375.

Centers for Disease Control (1987). Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR, 38:229-236.

Centers for Disease Control (1989). Update: Acquired immunodeficiency syndrome United States, 1981-1988. MMWR, 38:229-236.

Chandwani D, Borkowsky W, Krasinski K, Lawrence R, Bebenroth D, Moore T, (1987). Clinical features of respiratory synctial virus infection in human immunodeficiency virus infected children. III International AIDS Symposium, Washington, DC, p. 141.

Chilcote R, William SS, (1989). Therapy of Burkitt's lymphoma in children with infection. Pediatr Res, 25:149A, (abstr 876).

Chinn DH, (1988). Ultrasound evaluation of hydrops fetalis in Callen PW: Ultrasonography in Obstetrics and Gynecology (ed 2). Philadelphia: W.B. Saunders Company, pp277-298.

Church JA, Isaacs H, Saxon A, Keens TG, Richards W, (1981). Lymphoid interstitial pneumonitis and hypogammaglobulinemia in children. Am Rev Respir Dis, 124:491-496.

Cleary ML, Nalesnik MA, Shearer WT, et al (1988). Clonal analysis of transplant-associated lymphoproliferations based on the structure of the of the Epstein-Barr virus. Blood, 72:349-352.

Clement MJ, Luce JM, Hopewell PC, (1988). Diagnosis of pulmonary diseases. Clin Chest Med, 9:497-505.

Coates G, O'Brodovich H, (1986). Measurement of pulmonary epithelial permeability with Tc99m-DTPA aerosol. Semin Nuc Med, 16:275-284.

Cocchi P, Calabri G, Salvi G, et al (1988). AIDS-associated lymphoma of the brain in a child. Pediatr, 82:678.

Cohen IS, Anderson DW, Virmani R, et al (1986). Congestive cardiomyopathy in association with the acquired immunodeficiency syndrome. N Engl J Med, 315:628-630.

Cohen PR, Beltrani VP, Grossman ME, (1988). Disseminated herpes zoster in patients with human immunodeficiency virus infection. Am J Med, 84:1076-1080.

Colan SD, Borow KM, MacPherson D, Sanders SP, (1984). Use of the indirect axillary pulse tracing for noninvasive determination of ejection time, upstroke time, and left ventricular wall stress throughout ejection in infants and young children. Am J Cardiol, 53:1154.

Colan SD, Borow KM, Neumann A, (1985). Use of the calibrated carotid pulse tracing for calculation of left ventricular pressure and wall stress throughout ejection. Am Heart J, 109:1306.

Colan SD, Fujii A, Borow KM, MacPherson D, Sanders SP, (1983). Noninvasive determination of systolic, diastolic and end-systolic blood pressure in neonates, infants and young children: comparison with central aortic pressure measurements. Am J Cardiol, 52:867.

Colan SD, Borow KM, Neumann A, (1984). Left ventricular end-systolic wall stress-velocity of fiber shortening relations: a load-independent index of myocardial contractility. J Am Coll Cardiol, 4:715.

Colan SD, Sanders SP, Borow KM, (1987). Physiologic hypertrophy: effects on left ventricular systolic mechanics in athletes. J Am Coll Cardiol, 9:776.

Colan SD, Sanders SP, Inglefinger JR, Harmon W, (1987). Left ventricular mechanics and contractile state in children and young adults with end-stage renal disease: effect of dialysis and renal transplantation. J Am Coll Cardiol, 10:1085.

Coleman DL, Hattner RS, Luce JM, Dodek PM, Golden JA, Murray JF, (1984). Correlation between gallium lung scans and fiberoptic bronchoscopy in patients with suspected Pneumocystis carinii pneumonia and the acquired immune deficiency syndrome. Am Rev Respir Dis, 130:1166-1169.

Colin AA, Wohl MEB, Mead J, Ratjen FA, Glass G, Stark AR, (1989). Transition from dynamically maintained to relaxed end-expiratory volume in human infants. J Appl Physiol, In Press.

Cook NR, Ware JH, (1983). Design and Analysis Methods for Longitudinal Research. Ann Rev Pub Heal, 4:1-23.

Corallo S, Mutinelli MR, Moroni M, et al (1987). Echocardiography detects cardiac involvement in acquired immunodeficiency syndrome (AIDS): Study in 70 patients (abstr) in Proceedings of the Third International Conference on AIDS. Washington, DC, U.S. Department of Health and Human Services, THP-165.

Cowan MJ, Hellman D, Chudwin D, et al (1984). Maternal transmission of acquired immune deficiency syndrome. Pediatrics, 73:382-386.
Cox DR, (1972). Regression models and life tables. J Roy Stat Soc, 34B:187-220.

Curtis J, Goodman P, Hopewell P, (1986). Noninvasive tests in the diagnostic evaluation for p. carinii pneumonia in patients with or suspected of having AIDS. Am Rev Respir Dis, 133 Supplement: A182.

Damsker B, Bottone EJ, (1985). Mycobacterium avium - Mycobacterium intracellulare from the intestinal tracts of patients with the acquired immunodeficiency syndrome: Concepts regarding acquisition and pathogenesis. J Infect Dis, 151:179-181.

De Smedt MCH, Visser GHA, Meijboom EJ, (1987). Fetal cardiac output estimated by Doppler echocardiography during mid and late gestation. Am J Cardiol, 60:338-342.

Devereux RB, Reichek N, (1977). Echocardiographic determination of left ventricular mass in man. Circulation, 55:613-618.

DeVore GR, Siassi B, Platt LD, (1984). Fetal echocardiography. IV. M-mode assessment of ventricular size and contractility during the second and third trimesters of pregnancy in the normal fetus. Am J Ocstet Gynecol, 150:981.

Dittrich H, et al (1988). Human immunodeficiency virus, Coxsackie virus and cardiomyopathy. Ann Int Med. 108: 308-309.

Edelson JD, Hyland RH, (1989). Pulmonary complications of AIDS: a clinical strategy. CMAI, 140:1281-1287.

Ellaurie M, Wiznia A, Bernstein L, et al (1989). Lymphoma in pediatric HIV infection. Pediatr Res, 25:150A (abstr 884).

Engleberg LA, Lerner CW, Tapper ML, (1984). Clinical Commentary: Clinical features of pneumocystis pneumonia in the acquired immune deficiency syndrome. Am Rev Resp Dis, 130:689-694.

Epstein LG, DiCarlo EJ, Joshi VV, et al (1988). Primary lymphoma of the central nervous system in children with acquired immunodeficiency syndrome. Pediatrics, 82:355-363.

Fackler JC, Nagel JE, Adler WH, et al (1985). Epstein-Barr virus infection in a child with acquired immune deficiency syndrome. Am J Dis Ch, 139:1000.

Fallon J, Eddy J, Wiener L, et al (1989). Human immunodeficiency virus infection in children. J Pediatr, 114:1-30.

Fanconi S, (1988). Reliability of pulse oximetry in hypoxic infants. J Pediatr, 112:424-427.

Feltes TF, Hansen TN, (1986). Effects of a large aorticopulmonary shunt on lung fluid balance in newborn lambs. Pediatr Res, 20:368A.

Fink L, Reichek N, Sutton M, (1984). Cardiac abnormalities in acquired immune deficiency syndrome. Am J Cardiol, 54:1161-1163.

Fleiss JL, Tytun A, Ury, HK (1980). A simple approximation for calculating sample size for comparing independent proportions. Biometrics, 36:343-346.

Friedman-Kien AE, Lafleur FL, Gendler E, et al (1986). Herpes zoster: a possible early clinical sign for acquired immunodeficiency syndrome in high-risk individuals. J Am Acad Dermatol, 14:1023-1028.

Frishman W, Draus ME, Zabkar J, et al (1977). Infectious mononucleosis and fatal myocarditis. Chest, 72:535.

Frizzera G, Hanto DW, Gajl-Peczalska KJ, et al (1981). Polymorphic diffuse B-cell hyperplasias and lymphomas in renal transplant recipients. Cancer Res, 41:4262-4279.

Frizzera G, Rosai J, Dehner LP, et al (1980). Lymphoreticula disorders in primary immunodeficiencies. Cancer, 46:692-699.

Garson A, (1983). The electrocardiogram in infants and children: A systemic approach. Lea & Febiger, Philadelphia pp 195-343.

Gaultier C, (1985). Respiration au cours du sommeil pendant la croissance: physiologie et pathologie. Bull Europ Physio Path, 21:55-112.

Gerhardt T, (1985). A simple method for measuring functional residual capacity by N_2 washout in small animals and newborn infants. Pediatr Res, 19:1165-1169.

Godfrey S, Bar-Yishay E, Arad I, et al (1983). Flow volume curves in infants with lung disease. Pediatr, 72:517-522.

Gold JW, (1988). Overview of infection with the human immunodeficiency virus: infectious complications. Clin Chest Med, 9:377-386.

Goldberg SJ, Allen HD, Marx GR, Flinn CJ, (1985). Flow Computation in Goldberg SJ: Doppler Echocardiography. Lea & Febiger, Philadelphia pp 68-91.

Gottlieb MS, Schroff R, Schanker HM, et al (1981). Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: Evidence of a new acquired cellular immunodeficiency. N Engl J Med, 305:1425-1431.

Graybill JR, (1988). Histoplasmosis and AIDS. J Infect Dis, 158:623-626.

Guarner J, Brynes RK, Chan WC, (1987). Primary Non-Hodgkin's lymphoma of the heart in two patients with the acquired immunodeficiency syndrome. Arch Pathol Lab Med, 111:254-256.

Hatle L, Angelsen B, (1985). Doppler Ultrasound in Cardiology: Physical Principles and Clinical Application. 2nd Edition, Philadelphia, Lea & Febiger.

Hawkins CC, Gold JW, Whimbey E, et al (1988). Mycobacterium avium complex infections in patients with the acquired immunodeficiency syndrome. Ann Intern Med, 105:184-188.

Heaf DP, Turner H, Stocks J, Helms P, (1986). The accuracy of esophageal pressure measurements in convalescent and sick intubated infants. Ped Pulmonol, 2:5-8.

Henry WL, et al (1980). Report of the American Society of Echocardiography Committee on Nomenclature and Standards in two-dimensional echocardiography. Circulation, 62:212.

Hiatt P, (1988). Bronchodilator responsiveness in infants and young children with cystic fibrosis. Amer Rev Resp Dis, 137:119-122.

Holford TR, (1980). The analysis of rates and survivorship using log-linear models. Biometrics, 36:229-306.

Hopewell PC, Luce JM, (1985). Pulmonary involvement in the acquired immunodeficiency syndrome. Clin. Chest, 87:104-112.

Horsburgh Jr CR, Selik RM, (1989). The epidemiology of disseminated nontuberculous mycobacterial infection in the acquired immunodeficiency syndrome (AIDS). Am Rev Resp Dis, 139:4-7.

Issenberg HJ, Charytan M, Rubinstein A, (1985). Cardiac involvement in children with acquired immune deficiency (abstract). Am Heart J, 110:710.

Jacobson MA, Gillerman H, Chambers H, (1988). Staphylococcus aureus bacteremia and recurrent staphylococcal infection in patients with acquired immunodeficiency syndrome and AIDS-related complex. Am J Med, 85:172-176.

Jacobson MA, Mills J, (1983). Serious cytomegalovirus disease in acquired immunodeficiency syndrome (AIDS). Ann Intern Med, 108:585-594.

Jennrich RI, Schluchter MD, (1986). Unbalanced repeated measures models with structured covariance matrices. Biometrics, 42:805-820.

Jentry P, Roderick E, Delbeke D, et al (1984). Estimation of gestational age from measurement of fetal long bones. Jrn of Ultrasound in Medicine, 3:75.

Johnson PC, Khardori N, Najjar AF, et al (1988). Progressive disseminated histoplasmosis in patients with acquired immunodeficiency syndrome. Am J Med, 85:153-158.

Johnson JP, Prasanna N, Hines SE, et al (1989). Natural history and serologic diagnosis of infants born to human immunodeficiency virus - infected women. Amer J Dis Child, 143:1147-1153.

Joshi VV, Gadol C, Connor E, et al (1988). Dilated cardiomyopathy in children with acquired immunodeficiency syndrome: A pathological study of 5 cases. Hum Pathol, 19:69.

Joshi VV, Kauffman S, Oleske JM, et al (1987). Polyclonal polymorphic B-cell lymphoproliferative disorder with prominent pulmonary involvement in children with acquired immunodeficiency syndrome. Cancer, 59:1455.

Joshi VV, Oleske JM, (1986). Pulmonary lesions in children with the acquired immunodeficiency syndrome: A reappraisal based on data in additional cases and follow-up study of previously reported cases (letter). Hum Pathol, 17:641.

Joshi VV, Oleske JM, Minnefor AB, et al (1985). Pathologic pulmonary findings in children with the acquired immunodeficiency syndrome: a study of ten cases. Hum Pathol, 16:241-246.

Joshi VV, Oleske JM, Minnefor AB, Singh R, Bokhari T, Rapkin RH, (1984). Pathology of suspected acquired immune deficiency syndrome in children: a study of eight cases. Pediatr Pathol, 2:71-87.

Kamani N, Kennedy J, Brandsma J, (1988). Burkitt lymphoma in a child with human immunodeficiency virus infection. J Pediatr, 112:241-244.

Kaplan E, Meier P, (1958). Nonparametric estimation from incomplete observations. J Am Stat Assoc, 8:699-712.

Katz BZ, Andiman WA, Eastman R, Martin K, Miller G, (1986). Infection with two genotypes of Epstein-Barr virus in an infant with AIDS and lymphoma of the central nervous system. J Infect Dis, 153:601-604.

Kovacs JA, Ng VL, Masur H, et al (1988). Diagnosis of Pneumocystis carinii pneumonia: Improved detection in sputum with use of monoclonal antibodies. N Engl J Med, 318:589-653.

Krasinski K, Borkowsky W, Bonk S, et al (1988). Bacterial infections in human immunodeficiency virus-infected children. Pediatr Infect Dis J, 7:323-328.

Laird NM, Oliver D, (1981). Covariance analysis of censored survival data using log-linear analysis techniques. J of Amer Statistical Assoc, 76:231-240.

LeSouef PN, (1986). Effect of compression pressure on forced expiratory flow in infants. J Appl Physiol, 61:1639-1646.

LeSouef PN, England SJ, Bryan AC, (1984A). Passive respiratory mechanics in newborns and children. Am Rev Resp Dis, 129:552-556.

LeSouef PN, England SJ, Bryan AC, (1984B). Total resistance of the respiratory system in preterm infants with and without an endotracheal tube. J Pediatr, 104:108-111.

Lewis W, Lipsick J, Cammarosano C, (1985). Cryptococcal myocarditis in acquired immune deficiency syndrome. Am J Cardiol, 55:1240.

Liang KY, Zeger SL, (1986). Longitudinal data analysis using generalized linear Models. Biometrika, 73:13-22.

Lim W, Kahn E, Gupta A, et al (1988). Treatment of cytomegalovirus enterocolitis with ganciclovir in an infant with acquired immunodeficiency syndrome. Pediatr Infect Dis J, 7:354-357.

Lipshultz S, Chanock S, Sanders SP, Colan SD, McIntosh K, (1989). Cardiac manifestations of human immunodeficiency virus infection in infants and children. Am J Cardiol, 63:1489-1497.

Lipshultz SE, Chanock S, Sanders SP, Colan SD, Perez-Atayde A, McIntosh K, (1987). Cardiac manifestations of pediatric human immunodeficiency virus (HIV) infections. Circulation, 76:IV-515.

Lipshultz SE, Colan SD, Sanders SP, Sallan SE, (1988). Late cardiac effects of doxorubicin in childhood acute lymphoblastic leukemia. J Am Coll Cardiol, 11:135A.

Lipshultz SE, Sanders SP, Colan SD, McIntosh K, (1989B). Enchanced cardiac function pediatric HIV infection. Proc V Conf AIDS, TBP-146:311.

Lipshultz SE, Sanders SP, Colan SD, McIntosh K, (1989A). Enhanced LV function in pediatric human immunodeficiency virus infection. Pediatr Res, 25:183A.

Lipshultz SE, Sanders SP, Colan SD, Perez-Atayde A, Fox CH, McIntosh K, Winter H, (1989C). Does the increased myocardial mass seen in pediatric human immunodeficiency virus infection of cardiocytes result in direct infection of cardiocytes. Circulation, (In Press).

Lipshultz SE, Sanders SP, Mayer J, Colan SD, Lock JE, (1988). Are cardiac catheterization and angiography necessary in the preoperative evaluation of ASD-primum. J Am Coll Cardiol, 11:373-378.

Longo DL, (1984). Acquired immunodeficiency syndrome: Epidemiologic, immunologic, and therapeutic considerations. Kaposi's sarcoma and other neoplasms. Ann Intern Med, 199:96-99.

Lynch DA, et al (In Press). High resolution cine CT in pediatric pulmonary disease. Am J Radiol.

Mantel N, (1966). Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemotherapy Rep, 50:163-170.

Mantel N, Byar DP, (1974). Evaluation of response time data involving transplant status an illustration using heart transplant data. J Am Stat Assoc, 6981-

Marolda J, Pace B, Bonforte RJ, Cotin N, Kattan M, (In Press). Outcome of mechanical ventilation in children with acquired immunodeficiency syndrome. Pediatr Pulmonol.

Marquart KH, Muller HA, Hartter P, et al (1987). Lymphadenopathic type of Kaposi's sarcoma in an Ugandan child seropositive for LAV/HTLV-III antibodies. J Trop Med Hyg, 90(2):93-94.

Masters IB, (1987). Longitudinal study of lung mechanics in normal infants. Ped Pulmon, 3:3-7.

Maxfield RA, Sorkin IB, Fazzini EP, et al (1988). Respiratory failure in patients with acquired immunodeficiency syndrome and Pneumocystis carinii pneumonia. Critical Care Medicine, 14:443-449.

McCloud TC, et al (1983). Diffuse infiltrative lung disease: a new scheme for description. Radiology, 149:353-363.

Meignan M, Denis M, Autran B, (1988). Increased alveolar permeability (A.P) in HIV-infected patients with cytotoxic alveolities. Amer Rev Resp Dis, 137 (Suppl):358.

Meijboom EJ, Horowitz S, Valdes-Cruz LM, et al (1985). A Doppler echocardiographic method for calculating volume flow across the tricuspid valve: correlative laboratory and clinical studies. Circulation, 71:551-556.

Metz CE, (1978). Basic principles of ROC analysis. Seminars in Nuclear Med, 8:283-298.

Milner AD, Saunders RA, Hopkin IE, (1978). Tidal pressure volume and flow volume respiratory loop patterns in human neonates. Clin Sci & Molec Med, 54:257-264.

Morbidity and Mortality Weekly Report, (1987). Massachusetts Medical Society, Vol. 36, No. 15, Pages 225-236.

Morgan WJ, Geller DE, Tepper RS, Taussig LM, (1989). Partial expiratory flow-volume curves in infants and young children. Pediatr Pulmonol, 5:232-243.

Mortola JP, Fisher JT, Smith B, Fox G, Weeks S, (1982). Dynamic of breathing in infants. J Appl Physiol, 52:1209-1215.

Murphy PM, Lane CH, Fauci AS, Gallin TI, (1988). Impairment of neutrophil bactericidal capacity in patients with AIDS. J Infect Dis, 158:627-630.

Murray JF, Felton CP, Garay SM, et al (1984). Pulmonary complications of the acquired immunodeficiency syndrome. N Engl J Med, 310:1682-1688.

Nellcor, (1986). Controlling the external optical interference in pulse oximetry. pp 1-3, Nellcor Incorporated, Hayward California.

Nelson DL, Greene WC, Leonard WJ, Depper JM, Waldmann TA, (1986). The human interleukin-2 receptor: normal and abnormal expression in T-cells and in leukemias induced by human T-lymphotrophic retroviruses. Ann Int Med, 105:560.

Niedt G, Shinella R, (1985). Acquired immunodeficiency syndrome: Clinical pathologic study of 56 autopsies. Arch Pathol Lab Med, 109:727-734.

O'Brodovich HM, Moser MM, Lawrence L, (1980). Familial lymphoid interstitial pneumonia: A long term follow-up. Pediatrics, 65:523-528.

Ochs HD, (1987). Intravenous immunoglobulin in the treatment of prevention of acute infections in pediatric acquired immunodeficiency syndrome patients. Pediatr Infect Dis J, 509-511.

O'Connel JB, Reap EA, Robinson JA, (1986). The effect of cyclosporine on acute murine Coxsackie B3 myocarditis. Circulation, 73:353-359.

O'Doherty MJ, Page CJ, Bardbeer CS, et al (1987). Alveolar permeability in HIV antibody positive patients with Pneumocystic carinii pneumonia. Genitourin Med, 63:268-270.

Oldham SA, Castillo M. Jacobson M, et al (1989). HIV-associated lymphocytic interstitial pneumonia: radiologic manifestation and pathological correlation. Thoracic Radiol, 170:83-87.

Oleske J, Minnefor A, Cooper R, et al (1983). Immune deficiency syndrome in children. JAMA, 249:2345-2349.

Ott WJ, (1984). The use of ultrasonic fetal head circumference for predicion of estimated date of confinement. Jrn of Clinical Ultrasound, 12:411.

Packer SJ, Cesario T, Williams Jr JH, (1988). Mycobacterium avium complex infection presenting as endobronchial lesions in immunosuppressed patients. Ann Intern Med, 7:S61-S71.

Pahwa S, (1988). Human immunodeficiency virus infection in children: nature of immunodeficiency, clinical spectrum and management. Pediatr Infect Dis, 109:389-393.

Pahwa S, Kaplan M, Fikring S, et al (1986). Spectrum of human T-cell lymphotropic virus type III infection in children. J Am Med Assoc, 255:2299-2305.

Patrick CC, Hawkins EP, Guerra IC, et al (1987). A patient with leukemia in remission and acute abdominal pain. J Pediatr, 111:624-629.

Patton DF, Sixbey JW, Murphy SB, (1988). Epstein-Barr virus in human immunodeficiency virus-related Burkitt lymphoma. J Pediatr, 113:951-952.

Perera DR, Western KA, Johnson HD, et al (1970). Pneumocystis carinii pneumonia in a hospital for children. Epidemiologic aspects. JAMA, 214:1074-1078.

Pervez NK, Kleinerman J, Kattan M, et al (1985). Pseudomembanous necrotizing bronchial aspergillosis: A variant of invasive aspergiollosis in a patient with hemophilia and with aquired immunodeficiency syndrome. Amer Rev Resp. Dis, 131:961-963.

Peto R, Pike MC, Armitage P, et al (1977). Design and Analysis of Randomized Clinical Trials Requiring Prolonged Observations of each Patient. II. Analysis and Examples. Br J Cancer, 35:1.

Pitchenik AE, Fertel D, Bloch AB (1988). Mycobacterial disease: epidemiology, diagnosis, treatment, and prevention. Clin Chest Med, 9:425-441.

Praud JP, Carolfilis A, Bridey F, Lacaille F, et al (1989). Accuracy of two wavelength pulse oximetry in neonates and infants. Pediatr Pulmonol, 6:180-182.

Prose NS, Mendez H, Menikoff H, Miller HJ, (1987). Pediatric human immunodeficiency virus infection and its cutaneous manifestations. Pediatr Dermatol, 4:67-74.

Quinnan GV, Masur H, Rook AH, et al (1984). Herpes virus infections in the acquired immune deficiency syndrome. JAMA, 252:72-77.

Raab-Traub N, Flynn K, (1986). The structure of the termini of Epstein-Barr virus as a marker of clonal cellular proliferation. Cell, 47:883-889.

Rabin BS, (1983). Immunologic aspects of human cardiac transplantation. Heart Transplantation, II:188-191.

Rabinovitch M, Keane JF, Fellows KE, et al (1981). Qualitative analysis of the pulmonary wedge angiogram in congenital heart defects: Correlation with hemodynamic dates and morphometric findings in lung biopsy tissue. Circulation, 63:152-164.

Rahimi SA, (1974). Disseminated Pneumocystis carinii in thymic alymphoplasia. Arch Pathol, 97:162-165.

Ramanathan R, Durand M, Larrazabai C, (1987). Pulse oximetry in very low birth weight infants with acute and chronic lung disease. Pediatric, 79:612-617.

Rechavi G, Ben-Bassat I, Berkowicz M, et al (1987). Molecular analysis of Burkitt's leukemia in two hemophilic brothers with AIDS. Blood, 70:1713-1717.

Reed KL, Meijboom EJ, Sahn DJ, et al (1986). Cardiac Doppler flow velocities in human fetuses. Circulation, 73:41-46.

Reed KL, Sahn DJ, Scagnelli S, et al (1986). Doppler echocardiographic studies of diastolic function in the human fetal heart: Changes during gestation. J Am Coll Cardiol, 8:391.

Reitano J, King M, Cohen H, et al (1984). Cardiac function in patients with acquired immune deficiency syndrome (AIDS) or AIDS prodrome. J Am Coll Cardiol, 3:525.

Reynolds EOR, (1963). The effect of breathing 40 percent oxygen on the arterial blood gas tension of babies with bronchiolitis. J Pediatric, 63:1135.

Rogers MF, (1985). AIDS in children: a review of the clinical, epidemiologic and public health aspects. Pediatr Infect Dis, 4:230-236.

Rogers MF, (1988). Pediatric HIV infection epidemiology, etiopathogenesis, and transmission. Pediatr Ann, 17:324-331.

Rogers MF, Thomas PA, Starcher ET, et al (1987). Acquired immunodeficiency syndrome in children: Report of the Centers for Disease Control National Surveillance 1982-1985. Pediatrics, 79:1008-1014.

Ronchetti R, (1975). An analysis of a rebreathing method for measuring lung volume in the premature infant. Pediatr Res, 9:797-802.

Rook AH, (1988). Interactions of cytomegalovirus with the human immune system. Rev Infect Dis, 10:S460-S467, (Supp 3).

Rosenblatt HM, Lewis DE, Sklar J, et al (1987). Epstein-Barr virus transformed B-cell line (DV-1) derived from bone marrow of a patient with severe combined immunodeficiency and immunoblastic lymphoma. Pediatr Res, 21:331-337.

Rubin LA, Kurman CC, Fritz ME, Biddison WE, Yarchoan R, Nelson DL, (1985). Soluble interleukin-2 receptors are released from activated human lymphoid cells in vitro. J Immunol, 135:3172.

Rubinstein A, Bernstein L, (1986). The epidemiology of pediatric acquired immunodeficiency syndrome. Clin Immunol Immunopathol, 40:115-121.

Rubinstein A, Morecki R, Goldman H, (1988). Pulmonary disease in infants and children. Pulmonary effects of AIDS. Clin Chest Med, 9:507-516.

Rubinstein A, Morecki R, Silverman B, et al (1986). Pulmonary disease in children with acquired immune deficiency syndrome and AIDS related complex. J Pediatr, 108:498-503.

Rubinstein A, Sicklick M, Gupta A, et al (1983). Acquired immunodeficiency with reversed T4/T8 ratios in infants born to promiscuous and drug-addicted mothers. JAMA, 249:2350-2356.

Ryder RW, Nsa W, Hassig SE, et al (1989). Perinatal transmission of the human immunodeficiency virus type 1 to infants of seropositive women in Zaire. N Engl J Med, 320:1637-1642.

Schaeffer D, Cerny F, (1985). Effects of chloral hydrate on passive respiratory mechanics in infants. Physiologist, 28:303A, (Abstract)

Schluchter MD, (1988). Analysis of incomplete multivariate data using linear models with structured covariance matrices. Statistics in Medicine, 7:317-324.

Schluchter MD, Jackson KL, (1989). Log linear analysis of censored survival data with partially observed covariates. J Amer Stat Assoc, 84:42-52.

Schmidt KG, Birk E, Silverman NH, Scagnelli SA, (1989). Echocardiographic evaluation of dilated cardiomyopathy in the human fetus. Am J Cardiol, 63:599-605.

Schulman H, Fleischer A, Stern W, et al (1984). Umbilical velocity wave ratios in human pregnancy. Am J Obstet Gynecol, 148:985.

Scott GB, Buck BE, Leterman JG, et al (1984). Acquired immunodeficiency syndrome in infants. N Engl J Med, 310:76-81.

Shannon KM, Ammann AJ, (1985). Acquired immunodeficiency in Childhood. J Peds, 106:332-342.

Shapiro RS, McClain KL, Frizzera G, et al (1988). Epstein-Barr virus associated B-cell lymphoproliferative disorders following bone marrow transplantation. Blood, 71:1234-1243.

Sherron P, Pickoff A, Ferrer P, et al (1985). Echocardiographic evaluation of myocardial function in pediatric AIDS patients (abstract). Am Heart J, 110:710.

Shime J, Gresser CD, Rakowski H, (1986). Quantitative two-dimensional echocardiographic assessment of fetal cardiac growth. Am J Obstet Gynecol, 154:294-300.

Silver MA, Macher AM, Reichert CM, et al (1984). Cardiac involvement by Kaposi's sarcoma in acquired immune deficiency syndrome (AIDS). Am J Cardiol, 53:983-985.

Simpson H, Matthew DJ, Inglis JM, George EL, (1974). Virological findings and blood gas tensions in acute lower respiratory tract infections in children. Br Med J, 2:629.

Solal-Celigny P, Couderc LJ, Herman D, et al (1985). Lymphoid interstitial pneumonitis in acquired immunodeficiency syndrome-related complex. Am Rev Respir Dis, 131:956-960.

Steinherz LJ, Brochstein JA, (1986). Cardiac involvement in congenital acquired immunodeficiency syndrome. Am J Dis Child, 140:1241-1244.

Stewart JM, Kaul A, Gromisch DS, et al (1989). Symptomatic cardiac dysfunction in children with human immunodeficiency virus infection. Am Heart J, 117:140-144.

Stover DE, Meduri GU, (1988). Pulmonary function tests. Clin Chest Med, 9:473:479.

Stover DE, White DA, Romano PA, Gellene RA, Robeson WA, (1985). Spectrum of pulmonary diseases associated with the acquired immune deficiency syndrome. Am J Med, 78:429-437.

Strohl KP, House PM, Holic JF, Fouke JM, et al (1986). Comparison at three transmittance oximeters. Med Instrum, 20:143-149.

Stuart B, Drumm J, Fitzgerald DE, et al (1980). Fetal blood velocity waveforms in normal pregnancy. Br J Obstet Gynacol, 87:780.

Swedlow DB, Stern S, (1983). Continuous non-invasive oxygen saturation monitoring in children with a new pulse oximeter. Critical Care, 228.

Taussig L, Landau L, Godfrey S, (1982). Determinants of forced expiratory flow in newborn infants. J Appl Physiol, 53:1220-1227.

Taylor MD, Whitman JG, (1986). The current status of pulse oximetry. Anaesthesia, 41:943-949.

Tepper RS, Hiatt PW, (1988). Pulmonary function at diagnosis in infants with cystic fibrosis. Pediatr Pulmonol, 5:15-18.

Tepper RS, Hiatt PW, Eigen H, (1987). Total respiratory system compliance in asymptomatic infants with cystic fibrosis. Am Rev Respir Dis, 135:1075.

Tepper RS, Pagtakhan RD, Taussig LM, (1984). Noninvasive determination of total respiratory system compliance in infants by the weighted-spirometer method. Am Rev Respir Dis, 130:461-466.

Thomas PA, Jaffe HW, Spira TJ, et al (1984). Unexplained immunodeficiency in children: A surveillance report. JAMA, 252:639-644.

Tilney NL, Strom TB, Kupiec-Weglinski JW, (1987). Humoral and cellular mechanisms in acute allograft injury. J Pediatr, 111:1000-1003.

Tremper KK, (1989). Pulse Oximetry. Chest, 95:713-715.

Trudinger BJ, (1987). The umbilical circulation. Semin Perinatal, 11:311-321.

Trudinger BJ, Giles WB, Cook CM, (1985). Uteroplacental blood flow velocity-time waveforms in normal and complicated pregnancy. Br Obstet Gynaecol, 92:39.

Trudinger BJ, Giles WB, Cook CM, et al (1985). Fetal umbilical artery flow velocity waveforms and placental resistance: clinical significance. Br J Obstet Gynaecol, 92:23-30.

Vernon DD, Holzman BH, Lewis P, Scott GB, Birriel JA, Scott MB, (1988). Respiratory failure in children with acquired immunodeficiency syndrome and acquired immunodeficiency syndrome-related complex. Pediatrics, 82:223-228.

Victoria MS, Nangia BS, Jindrak K, (1985). Cytomegalovirus pyloric obstruction in a child with acquired immunodeficiency syndrome. Pediatr Infect Dis, 4:550-552.

Vogel RL, Alboliras ET, McSherry O, Levine R, Antillon JR, (1988). Congenital heart defects in children of human immunodeficiency virus positive mothers. Circulation, 78 (2) 17.

Ware JH, (1985). Linear Models for the Analysis of Longitudinal Studies. Am Statistician, 39:95-101.

Welch K, Finkbeiner W, Alpers CE, (1984). Autopsy findings in the acquired immune deficiency syndrome. JAMA, 252:1152-1159.

Wheat LJ, Rubin RH, Harris NL, et al (1987). Systemic salmonellosis in patients with disseminated histoplasmosis. Case of macrophage blockage caused by Histoplasma capulatum. Arch Intern Med, 147:561-564.

Wheat LJ, Salma TG, Zechkel ML, (1985). Histoplasmosis in the acquired immune deficiency syndrome. Am J Med, 78:203-210.

Wink K, Schmitz, (1980). Cytomegalovirus myocarditis. Am Heart J, 100:667.

Wu MC, Bailey KR, (1988). Analyzing Changes in the presence of informative right censoring caused by death and withdrawal. Stat in Medicine, 7:337-346.

Wu MC, Bailey KR, (1989). Estimation and comparison of changes in presence of informative right censoring: conditional linear model. Biometrics, 45:939-955.

Wu MC, Carroll RJ, (1988). Estimation and comparison of changes in the presence of informative right censoring by modeling the censoring process. Biometrics, 44:175-188.

Yelderman M, New Jr W, (1983). Evaluation of pulse oximetry. Anesthesiology, 59:349-352.

Young LS, (1988). Mycobacterium avium complex infection. J Infect Dis, 157:863-867.

Young LS, Inderlied CB, Berlin OG, Gottlieb MS, (1986). Mycobacterial infections in AIDS patients with an emphasis on the Mycobacterium avium complex. Rev Infect Dis, 8:1023-1033.

Zin WA, Pengelly LD, Milic-Emili J, (1982). Single breath method for measurement of respiratory mechanics in anesthetized animals. J Appl Physiol, 52:1266-1271.

APPENDIX 1

APPENDIX 1

Phase I: May 1, 1989 - June 15, 1990

(Develop the Protocol, Manual of Operations, Forms, Distributed Data Entry System and Train Personnel)

Contract for Clinical Coordinating Center Awarded ..... May 1, 1989

Contracts for Clinical Centers Awarded ..... May 22, 1989

First Steering Committee Meeting ..... May 30–31, 1989

First Draft of Protocol Submitted to the Policy, Data and Safety Monitoring (PDSM) Board ..... October 16, 1989

First PDSM Board Meeting ..... November 17, 1989

First Draft of Manual of Operations and Forms Submitted to the PDSM Board ..... February 19, 1990

Final Protocol, Manual of Operations, Forms and Database System Completed ..... June 15, 1990

Phase II: May 22, 1990 - January 31, 1997 (6 2/3 years)

Recruitment Begins ..... May 22, 1990

Training of Clinical Center Personnel ..... July 16, 1990

Recruitment Ends (original) ..... May 21, 1992

Recruitment Ends (Group II extension) ..... January 31, 1994

Follow–up Ends (original) ..... November 21, 1994

Follow–up Ends (Group I and Group II extension) ..... January 31, 1997

Phase III: August 1, 1996 - July 31, 1997

(As requested by the NHLBI, Phase III overlaps Phase II by six months, for analyses and manuscript preparation).

Final Report and Contract Termination ..... July 31, 1997

(Rev. 6/10/93)

APPENDIX 2

Employee Exposure to Human Immunodeficiency Virus
At Texas Children's Hospital
In Affiliation With Baylor College Of Medicine

Policy

It is the policy of Texas Children's Hospital to provide a safe working environment for all employees. Texas Children's Hospital will provide testing for HIV antibodies to any employee who suffers a parenteral (i.e. needle stick) or mucous membrane (i.e. mouth or eye) exposure to blood or body fluids containing blood from an HIV infected or possible HIV infected patient. Testing will be provided to any employee who otherwise suspects exposure to the HIV virus in the work environment.

Employment

1. An employee known to be infected with HIV will not be restricted from work unless:

   a. the employee has another illness or infection for which a restriction is indicated;

   b. the employee's duties require performance or participation in invasive procedures;

   c. it has been determined that the employee is susceptible to infections to which (s)he may come into contact in the performance of his/her duties.

   The job duties of any employee with a positive HIV antibody test will be reviewed by a hospital physician, the employee's administrator and the department head in order to determine the appropriateness of the employee's job assignment. The employee's physician may be consulted as part of the review process.

2. Care of the HIV Infected Patients

   Employees are expected to perform appropriate work duties in caring for HIV infected patients. Employees refusing to perform such duties will be subject to disciplinary action up to and including discharge.

3. Working with HIV Infected Co-Workers

   It is the policy of the Hospital to assure that employees are educated with respect to the transmission of HIV infections. Co-workers who refuse to work with an HIV infected co-worker will be subject to disciplinary action up to and including discharge.

Blood and Body Fluid Precautions

1. Employees should consider all patients to be potentially infected with HIV. Blood and body fluid precautions should be consistently used for all patients.

   a. Gloves will be available in all patient care areas and must be worn by all employees who come in contact with blood and/or body fluids containing gross blood, or non-intact skin or when handling items or surfaces soiled with blood or body fluids containing blood.

   b. Masks, protective eyewear and gowns will be worn during invasive or other procedures that are likely to generate splashes of blood or other body fluids containing blood.

   c. Mouthpieces, resuscitative bags or other ventilation devices will be made available in those areas where the need for resuscitation is predictable; i.e., crash carts.

Exposure of Employees to HIV

1. Procedures when source of exposure is known.

   a. Exposed employees should be advised to contact the Employee Health Department as soon as possible after exposure. All follow-up work is conducted or coordinated by the Employee Health Department.

   b. The Supervisor will document specific known exposures by completing an employee injury report.

   c. The Employee Health Nurse will explain HIV testing, protocols and procedures to the employee.

   d. The consent form for HIV testing must be signed by the employee prior to the testing.

   e. Specimens will be labeled with code number only. The employee's name will not appear on any laboratory test requisition or specimen.

   f. All test results will be maintained by the Employee Health Department in a privilege and confidential manner.

   g. It shall be the responsibility of the employee to contact the Employee Health Department 72 hours after the blood tests for information regarding results.

   h. If the test result is positive, the Employee Health Nurse will advise the employee health physician.

i. Employees who test positive will be informed as such, evaluated on an individual basis and counselled by the employee health physician.

j. In the event of a positive result, continued employment in the employee's present position will be evaluated by the employee health physician, the appropriate administrative level individual, and the employee's department head.

k. If the test results are negative, the employee will be retested in accordance with CDC guidelines at intervals of six weeks, three months, six months, and one year.

2. Procedures when source of exposure is unknown:

a. The employee should be advised to contact the Employee Health Department.

b. The Employee Health Nurse will document the exposure incident as described by the employee.

c. The HIV testing protocol and procedures will be explained by the Employee Health Nurse.

d. A consent form for HIV testing must be signed by the employee prior to testing.

e. Specimens will be labeled with code number only. The employee's name will not appear on any laboratory test requisition or specimen.

f. All test results will be maintained by the Employee Health Department in a privilege and confidential manner.

g. It shall be the responsibility of the employee to contact the Employee Health Department 72 hours after the blood tests for information regarding results.

h. If the test result is negative, no further action is indicated.

i. If the test result is positive, the Employee Health Nurse will advise the employee health physician.

j. Employees who test positive will be informed as such, evaluated on an individual basis and counselled by the employee health physician.

k. In the event of a positive result, continued employment in the employee's present position will be evaluated by the employee health physician, the appropriate administrative level individual, and the employee's department head.

Training of Employees

All employees and students in patient care areas will be trained on the epidemiology, modes of transmission and prevention of HIV and other blood born infections and the need for unusual blood and body fluid precautions for all patients.

Universal Precautions

Objective

To prevent the spread of unknown disease or infection.

Policies and General Information

1. Medical history and examination will not always identify those patients who are infected with the Human Immunodeficiency Virus (HIV) or other blood-borne pathogens, such as Hepatitis B. Therefore, Universal Precautions will be followed on all patients.

2. Barrier precautions will be followed on all patients.

3. Gloves will be worn by all Health Care Workers (HCW) when drawing blood, starting IV's and handling non-intact skin or items contaminated with blood or body fluids contaminated with blood on all patients.

4. Hands should be washed after all patient contact and after contact with surfaces or items contaminated with blood or body fluids containing blood. Hands should be washed after removing gloves.

5. Care should be taken to prevent accidental injuries from needlesticks or other sharps (scalpels). Needles should not be recapped, bent or broken, but should be placed in puncture proof containers.

6. Mouthpieces, resuscitation bags or other ventilation devices should be made available in those areas where need for resuscitation is predictable.

7. During the performance of invasive procedures which could generate droplet aerosolization of blood or body fluids contaminated with blood, those HCW's involved should wear impervious gowns, gloves, masks and protective eyewear.

8. Blood samples or other specimens visibly contaminated with blood should be placed in clear, plastic bags prior to transportation to the laboratory. There is no need to label any specimen as all specimens will be treated as potentially infectious.

9. Linens require no special handling unless there is gross contamination with blood or bloody drainage.

Policies & Procedures - HIV Exposure - Pathology

Based on both the universal and the hospital recommendations, the Department of Pathology is modifying its operating procedures to incorporate the following:

1. Gloves will be worn by phlebotomists when drawing blood specimens. When drawing from multiple patients, a new pair of gloves will be worn for each patient. Employees processing and analyzing blood, urine or other body fluids must wear gloves.

2. Hands must be washed after removing gloves.

3. Needles will not be recapped, bent or broken, but will be placed in puncture proof containers.

4. Specimen containers visibly contaminated with blood, urine or other body fluids will be placed in clear, plastic bags prior to transportation to the laboratory. After handling a contaminated container, employees must discard contaminated gloves for a new pair. With the implementation of universal precautions there is no need to label any specimen as "Infectious", as all specimens will be treated as potentially infectious.

5. Employees will be issued a first set of eyewear, additional eyewear must be purchased by the employee if lost or stolen. The eyewear is to be worn during procedures that are likely to generate splashes of blood or other body fluids.

6. When requested the department will furnish masks for the employees processing (centrifuging) the blood specimens or other body fluids.

7. Employees and other staff must wear gloves when handling telephones or other equipment located in areas where blood specimens or body fluids are processed or analyzed.

8. Employees must remove gloves and must wash hands prior to leaving their work area.

9. Access to the morgue is limited to those personnel directly involved in the area. All persons entering the morgue while a case is in progress must wear appropriate protective clothing, masks, and shoe covers. These must be removed prior to leaving the area.

APPENDIX 3

CENTERS FOR DISEASE CONTROL
PROSPECTIVE EVALUATION OF HOSPITAL PERSONNEL EXPOSED TO BLOOD FROM PATIENTS WITH AIDS VIA THE PARENTERAL ROUTE

Department of Health and Human Services
Public Health Service
Centers for Disease Control
Atlanta, Georgia 30333

OBJECTIVE:

The objective of this prospective surveillance system is to evaluate the occurrence of acquired immunodeficiency syndrome (AIDS) in hospital personnel who have been exposed by the parenteral or mucous membrane route to blood from patients with AIDS or possible AIDS.

MATERIALS AND METHODS

A. Enrollment

Personnel would be eligible for enrollment in this prospective surveillance system if they had been exposed to blood from a patient with AIDS or possible AIDS prodrome (e.g., chronic lymphadenopathy, wasting syndrome), if this exposure occurred by needlestick, by contamination of an open wound, by splash in the eye, or by ingestion. Major medical institutions which have reported AIDS cases will be asked to participate in this evaluation. Infectious disease specialists, the hospital epidemiologist or the Employee Health Service physician at these institutions will be enlisted as cooperating investigators. These investigators will be responsible for surveillance of personnel at their institution, for registering exposed personnel, and for data collection and prospective evaluation of exposed personnel for the duration of this observation period (see D, below).

Participation is voluntary. If required by the participating institution, informed consent will be obtained from the exposed personnel upon entry into this surveillance system.

B. Data Collection

Upon entry into the prospective surveillance system, each exposed employee will be interviewed by the institutional investigator and a questionnaire will be completed to collect data concerning the employee's past medical history, use of possible immunosuppressive medications, other risk factors which have been found to be associated with AIDS, the degree and type of exposure to the AIDS patient's blood, and the type of precautions used to prevent exposure, if any (PERSONNEL EXPOSURE REPORT FORM--INITIAL CASE REPORT FORM). A baseline physical examination and laboratory testing will be performed at this time.

The employee will be asked to complete questions concerning other possible risk factors for AIDS which are of a sensitive nature (QUESTIONNAIRE 2). QUESTIONNAIRE 2 will be completed by the employee and mailed directly by the employee to CDC in a pre-addressed envelope. During the surveillance period, the employee will be instructed to report any illness lasting longer than 1 week to the hospital investigator.

In addition, data will be collected concerning the patient to whom the employee was exposed, including the basis for the diagnosis of AIDS and the infections present at the time of the exposure. This data will be collected using the standard AIDS CASE REPORT FORM. If this form has already been completed for the AIDS patient to whom the employee was exposed and forwarded to the CDC or the State Health Department, it will not be necessary to complete another form.

A serum sample (10 ml.) and a heparinized whole blood sample (10 ml.) will be requested from the employee at the time of the initial interview. These specimens will be sent, at ambient temperature, within 24 hours after collection, by express mail to the CDC. The serum specimens will be banked at the CDC. This sera will be tested for serologic markers of AIDS at a future time, when an etiologic agent is identified. Tests for T-cell subsets will be performed on the whole blood specimen and the results forwarded to the institutional investigator periodically.

E. Records Management

Each participating institution will be assigned a center code number by the CDC. Each participating employee will be assigned an employee code number by the CDC. These code numbers will be pre-printed on the report forms. Only the code numbers will be used in reporting data or submitting specimens to the CDC, in order to protect confidentiality. The institutional investigator will be responsible for maintaining a record of the name and code number of exposed personnel. It will be necessary for the institutional investigator to have access to names of exposed employees because the institutional investigator will be responsible for coordinating continued follow-up of exposed employees should they leave the employ of their institution.

F. Data Analysis

The questionnaires will be analyzed to provide a descriptive profile of exposed personnel. If illness is identified in exposed personnel, further analysis will be done. Personnel who became ill will be compared with exposed personnel who did not become ill. Demographic data, the type of exposure to AIDS patients, and the type of precautions used, if any, will be analyzed. Data analysis will be performed in conjunction with Statistics and Computing Activity, Hospital Infections Program.

Summaries of all data collected will be provided to investigators from participating institutions on a periodic basis. Data will be summarized and reported regularly in the Morbidity and Mortality Weekly Report.

SUMMARY

This prospective surveillance system will determine the occurrence of AIDS in hospital employees who are exposed to potentially infectious blood via parenteral or mucous membrane routes. The appropriateness and effectiveness of precautions currently recommended for prevention can then be re-evaluated. Additional information can be expected from this prospective observation if an etiologic agent is found in the future. Banked serum can then be investigated for the incidence of subclinical infection and its incubation period using appropriate serological markers. Associated employee risk factors may be better defined.

Each institutional investigator will receive from CDC all forms necessary for registering exposed employees into the surveillance system. These include a PERSONNEL EXPOSURE REPORT FORM--INITIAL CASE REPORT FORM, QUESTIONNAIRE 2, an AIDS CASE REPORT FORM, an instruction form for submitting specimens, specimen labels, containers for shipment of specimens, pre-addressed and franked mailing labels for express mail, and pre-addressed and franked shipping envelopes.

C. Post-exposure Prophylaxis

A decision about prophylaxis for hepatitis B for the exposed employees will be made by the individual hospitals using previously published recommendations for hepatitis B immune globulin (HBIG) and hepatitis B vaccine. No data are available concerning the value of HBIG or other modes of prophylaxis or therapy (e.g., immune globulin) for AIDS exposure. Entry into this surveillance system should in no way influence therapeutic decisions if information about useful therapy or prophylaxis for AIDS becomes available during the observation period.

D. Follow-up

The exposed employees will be followed prospectively by the institutional investigator every six months for 3 years. At the time of each follow-up, a follow-up questionnaire and physical examination will be repeated (PERSONNEL EXPOSURE FOLLOW-UP FORM). Serum and whole blood will be drawn and sent to CDC; serum specimens will be banked and whole blood tested for T-cell subsets. Additional laboratory testing (chest x-rays, skin tests, white blood cell counts) may be performed at the time of follow-up, as deemed appropriate by the institutional investigator.

Shortly before the scheduled time for each follow-up evaluation, CDC will send to the institutional investigator a notification of the scheduled follow-up, a PERSONNEL EXPOSURE FOLLOW-UP FORM, specimen labels, containers for shipment of specimens, pre-addressed and franked mailing labels for express mail, and pre-addressed and franked shipping envelopes.

APPENDIX 4

Working Safely with HIV in the Research Laboratory

Biosafety Level 2/3

Occupational Safety and Health Branch
NIH Division of Safety

National Institutes of Health
Bethesda, Maryland 20892
Building : 31
Room : 1C02
(301) 496- 1357

JUN 2 1988

Dear Colleague:

The NIH Division of Safety has compiled this set of guidance documents for laboratory personnel working with human immunodeficiency viruses. Included are two issues of Morbidity and Mortality Weekly Report entitled, "1988 Agent Summary Statement for Human Immunodeficiency Virus and Report on Laboratory-Acquired Infection with Human Immunodeficiency Virus" (April 1, 1988/Vol. 37/No. S-4) and "Recommendations for Prevention of HIV Transmission in Health-Care Settings" (August 21, 1987/Vol. 36/No. 2S). Included as an adjunct to these articles is a copy of "Effectively Using Biological Safety Cabinets." The recommended facilities, practices and procedures for laboratory personnel working with HIV at biosafety level 2/3 and biosafety level 3 are incorporated within the text of the Agent Summary Statement.

Because the objectives, and therefore the laboratory procedures of research vary, it is incumbent upon all principal investigators and laboratory personnel to assess risk before initiating research. You are encouraged to review these documents and to use them as a framework from which to develop your own safety program to ensure that appropriate safety practices are adopted.

The Division of Safety is in the process of producing a videotape entitled, "Working Safely with HIV in the Research Laboratory" which visually depicts the same broad microbiological techniques described in these documents. It is intended to assist laboratory personnel, research administrators and safety professionals formulate questions that must be answered before research with HIV is started. I anticipate that the videotape will be ready for distribution in mid-June. By sending in the enclosed postcard your request for the videotape can be filled more quickly.

One copy of the tape will be made available to each institute free of charge. I would therefore encourage you to develop an internal duplication and distribution policy as a means to provide this vital information to others at your institution. You are welcome to announce its availability in an internal newsletter and provide as many duplicate copies as necessary to educate your laboratory personnel.

Thank you for your interest.

Sincerely,

Robert W. McKinney

Robert W. McKinney, Ph.D.
Director
Division of Safety

CENTERS FOR DISEASE CONTROL

April 1, 1988 / Vol. 37 / No. S-4

MMWR

Supplement

MORBIDITY AND MORTALITY WEEKLY REPORT

1988 Agent Summary Statement for Human Immunodeficiency Virus

and

Report on Laboratory-Acquired Infection with Human Immunodeficiency Virus

U.S. Department of Health and Human Services
Public Health Service
Centers for Disease Control
Center for Infectious Diseases
Hospital Infections Program
AIDS Program
Atlanta, Georgia

APPENDIX 5

August 14, 1987 / Vol. 36 / No. 1S

MORBIDITY AND MORTALITY
WEEKLY REPORT

Supplement

Printed and distributed by the Massachusetts Medical Society,
publishers of *The New England Journal of Medicine*

Revision of the CDC Surveillance Case Definition for Acquired Immunodeficiency Syndrome

AIDS Program
Center for Infectious Diseases
Centers for Disease Control
Atlanta, Georgia 30333

Revision of the CDC Surveillance Case Definition for Acquired Immunodeficiency Syndrome

Reported by
Council of State and Territorial Epidemiologists;
AIDS Program, Center for Infectious Diseases, CDC

INTRODUCTION

The following revised case definition for surveillance of acquired immunodeficiency syndrome (AIDS) was developed by CDC in collaboration with public health and clinical specialists. The Council of State and Territorial Epidemiologists (CSTE) has officially recommended adoption of the revised definition for national reporting of AIDS. The objectives of the revision are a) to track more effectively the severe disabling morbidity associated with infection with human immunodeficiency virus (HIV) (including HIV-1 and HIV-2); b) to simplify reporting of AIDS cases; c) to increase the sensitivity and specificity of the definition through greater diagnostic application of laboratory evidence for HIV infection; and d) to be consistent with current diagnostic practice, which in some cases includes presumptive, i.e., without confirmatory laboratory evidence, diagnosis of AIDS-indicative diseases (e.g., *Pneumocystis carinii* pneumonia, Kaposi's sarcoma).

The definition is organized into three sections that depend on the status of laboratory evidence of HIV infection (e.g., HIV antibody) (Figure 1). The major proposed changes apply to patients with laboratory evidence for HIV infection: a) inclusion of HIV encephalopathy, HIV wasting syndrome, and a broader range of specific AIDS-indicative diseases (Section II.A); b) inclusion of AIDS patients whose indicator diseases are diagnosed presumptively (Section II.B); and c) elimination of exclusions due to other causes of immunodeficiency (Section I.A).

Application of the definition for children differs from that for adults in two ways. First, multiple or recurrent serious bacterial infections and lymphoid interstitial pneumonia/pulmonary lymphoid hyperplasia are accepted as indicative of AIDS among children but not among adults. Second, for children<15 months of age whose mothers are thought to have had HIV infection during the child's perinatal period, the laboratory criteria for HIV infection are more stringent, since the presence of HIV antibody in the child is, by itself, insufficient evidence for HIV infection because of the persistence of passively acquired maternal antibodies < 15 months after birth.

The new definition is effective immediately. State and local health departments are requested to apply the new definition henceforth to patients reported to them. The initiation of the actual reporting of cases that meet the new definition is targeted for September 1, 1987, when modified computer software and report forms should be in place to accommodate the changes. CSTE has recommended retrospective application of the revised definition to patients already reported to health departments. The new definition follows:

1987 REVISION OF CASE DEFINITION FOR AIDS FOR SURVEILLANCE PURPOSES

For national reporting, a case of AIDS is defined as an illness characterized by one or more of the following "indicator" diseases, depending on the status of laboratory evidence of HIV infection, as shown below.

I. Without Laboratory Evidence Regarding HIV Infection

If laboratory tests for HIV were not performed or gave inconclusive results (*See* Appendix I) and the patient had no other cause of immunodeficiency listed in Section I.A below, then any disease listed in Section I.B indicates AIDS if it was diagnosed by a definitive method (*See* Appendix II).

A. **Causes of immunodeficiency that disqualify diseases as indicators of AIDS in the absence of laboratory evidence for HIV infection**
   1. high-dose or long-term systemic corticosteroid therapy or other immunosuppressive/cytotoxic therapy ≤3 months before the onset of the indicator disease
   2. any of the following diseases diagnosed ≤3 months after diagnosis of the indicator disease: Hodgkin's disease, non-Hodgkin's lymphoma (other than primary brain lymphoma), lymphocytic leukemia, multiple myeloma, any other cancer of lymphoreticular or histiocytic tissue, or angioimmunoblastic lymphadenopathy
   3. a genetic (congenital) immunodeficiency syndrome or an acquired immunodeficiency syndrome atypical of HIV infection, such as one involving hypogammaglobulinemia

B. **Indicator diseases diagnosed definitively (*See* Appendix II)**
   1. candidiasis of the esophagus, trachea, bronchi, or lungs
   2. cryptococcosis, extrapulmonary
   3. cryptosporidiosis with diarrhea persisting >1 month
   4. cytomegalovirus disease of an organ other than liver, spleen, or lymph nodes in a patient >1 month of age
   5. herpes simplex virus infection causing a mucocutaneous ulcer that persists longer than 1 month; or bronchitis, pneumonitis, or esophagitis for any duration affecting a patient >1 month of age
   6. Kaposi's sarcoma affecting a patient < 60 years of age
   7. lymphoma of the brain (primary) affecting a patient < 60 years of age
   8. lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia (LIP/PLH complex) affecting a child <13 years of age
   9. *Mycobacterium avium* complex or *M. kansasii* disease, disseminated (at a site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
   10. *Pneumocystis carinii* pneumonia
   11. progressive multifocal leukoencephalopathy
   12. toxoplasmosis of the brain affecting a patient >1 month of age

II. With Laboratory Evidence for HIV Infection

Regardless of the presence of other causes of immunodeficiency (I.A), in the presence of laboratory evidence for HIV infection (*See* Appendix I), any disease listed above (I.B) or below (II.A or II.B) indicates a diagnosis of AIDS.

A. Indicator diseases diagnosed definitively (*See* Appendix II)

1. bacterial infections, multiple or recurrent (any combination of at least two within a 2-year period), of the following types affecting a child < 13 years of age:

   septicemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ or body cavity (excluding otitis media or superficial skin or mucosal abscesses), caused by *Haemophilus, Streptococcus* (including pneumococcus), or other pyogenic bacteria
2. coccidioidomycosis, disseminated (at a site other than or in addition to lungs or cervical or hilar lymph nodes)
3. HIV encephalopathy (also called "HIV dementia," "AIDS dementia," or "subacute encephalitis due to HIV") (*See* Appendix II for description)
4. histoplasmosis, disseminated (at a site other than or in addition to lungs or cervical or hilar lymph nodes)
5. isosporiasis with diarrhea persisting >1 month
6. Kaposi's sarcoma at any age
7. lymphoma of the brain (primary) at any age
8. other non-Hodgkin's lymphoma of B-cell or unknown immunologic phenotype and the following histologic types:
   a. small noncleaved lymphoma (either Burkitt or non-Burkitt type) (*See* Appendix IV for equivalent terms and numeric codes used in the *International Classification of Diseases*, Ninth Revision, Clinical Modification)
   b. immunoblastic sarcoma (equivalent to any of the following, although not necessarily all in combination: immunoblastic lymphoma, large-cell lymphoma, diffuse histiocytic lymphoma, diffuse undifferentiated lymphoma, or high-grade lymphoma) (*See* Appendix IV for equivalent terms and numeric codes used in the *International Classification of Diseases*, Ninth Revision, Clinical Modification)

   Note: Lymphomas are not included here if they are of T-cell immunologic phenotype or their histologic type is not described or is described as "lymphocytic," "lymphoblastic," "small cleaved," or "plasmacytoid lymphocytic"
9. any mycobacterial disease caused by mycobacteria other than *M. tuberculosis*, disseminated (at a site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
10. disease caused by *M. tuberculosis*, extrapulmonary (involving at least one site outside the lungs, regardless of whether there is concurrent pulmonary involvement)
11. *Salmonella* (nontyphoid) septicemia, recurrent
12. HIV wasting syndrome (emaciation, "slim disease") (*See* Appendix II for description)

B. Indicator diseases diagnosed presumptively (by a method other than those in Appendix II)

Note: Given the seriousness of diseases indicative of AIDS, it is generally important to diagnose them definitively, especially when therapy that would be used may have serious side effects or when definitive diagnosis is needed

for eligibility for antiretroviral therapy. Nonetheless, in some situations, a patient's condition will not permit the performance of definitive tests. In other situations, accepted clinical practice may be to diagnose presumptively based on the presence of characteristic clinical and laboratory abnormalities. Guidelines for presumptive diagnoses are suggested in Appendix III.

1. candidiasis of the esophagus
2. cytomegalovirus retinitis with loss of vision
3. Kaposi's sarcoma
4. lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia (LIP/PLH complex) affecting a child <13 years of age
5. mycobacterial disease (acid-fast bacilli with species not identified by culture), disseminated (involving at least one site other than or in addition to lungs, skin, or cervical or hilar lymph nodes)
6. *Pneumocystis carinii* pneumonia
7. toxoplasmosis of the brain affecting a patient >1 month of age

III. With Laboratory Evidence Against HIV Infection

With laboratory test results negative for HIV infection (*See* Appendix I), a diagnosis of AIDS for surveillance purposes is ruled out *unless*:

A. all the other causes of immunodeficiency listed above in Section I.A are excluded; AND

B. the patient has had either:

1. *Pneumocystis carinii* pneumonia diagnosed by a definitive method (*See* Appendix II); OR

2. a. any of the other diseases indicative of AIDS listed above in Section I.B diagnosed by a definitive method (*See* Appendix II); AND

   b. a T-helper/inducer (CD4) lymphocyte count <400/mm^3.

COMMENTARY

The surveillance of severe disease associated with HIV infection remains an essential, though not the only, indicator of the course of the HIV epidemic. The number of AIDS cases and the relative distribution of cases by demographic, geographic, and behavioral risk variables are the oldest indices of the epidemic, which began in 1981 and for which data are available retrospectively back to 1978. The original surveillance case definition, based on then-available knowledge, provided useful epidemiologic data on severe HIV disease (*1*). To ensure a reasonable predictive value for underlying immunodeficiency caused by what was then an unknown agent, the indicators of AIDS in the old case definition were restricted to particular opportunistic diseases diagnosed by reliable methods in patients without specific known causes of immunodeficiency. After HIV was discovered to be the cause of AIDS, however, and highly sensitive and specific HIV-antibody tests became available, the spectrum of manifestations of HIV infection became better defined, and classification systems for HIV infection were developed (*2-5*). It became apparent that some progressive, seriously disabling, and even fatal conditions (e.g., encephalopathy, wasting syndrome) affecting a substantial number of HIV-infected patients were not subject to epidemiologic surveillance, as they were not included in the AIDS

Table of Contents

Agent Summary Statement for Human Immunodeficiency Viruses (HIVs) Including HTLV-III, LAV, HIV-1, and HIV-2*

INTRODUCTION

In 1984, the Centers for Disease Control (CDC) and the National Institutes of Health (NIH), in consultation with experts from academic institutions, industry, and government, published the book *Biosafety in Microbiological and Biomedical Laboratories* ("*Guidelines*")† (*1*).

These *Guidelines* are based on combinations of standard and special practices, equipment, and facilities recommended for use in working with infectious agents in various laboratory settings. The recommendations are advisory; they provide a general code for operating microbiologic and biomedical laboratories.

One section of the *Guidelines* is devoted to standard and special microbiologic practices, safety equipment, and facilities for biosafety levels (BSL) 1 through 4. Another section contains specific agent summary statements, each consisting of a brief description of laboratory-associated infections, the nature of laboratory hazards, and recommended precautions for working with the causative agent. The authors realized that the discovery of the availability of information about these agents would necessitate updating the agent summary. Such a statement for human immunodeficiency virus (HIV) (called HTLV-III/LAV when the *Guidelines* were published) was published in *MMWR* in 1986 (*2*). The HIV agent summary statement printed in this *Supplement* updates the 1986 statement.

Attached to the updated HIV agent summary statement are the essential elements for BSL 2 and 3 laboratories, reproduced from the *Guidelines* (*1*) (see Addendum 1, p. 6). BSL 2 and 3 laboratory descriptions are included because they are recommended for laboratory personnel working with HIV, depending on the concentration or quantity of virus or the type of laboratory procedures used.

*The information and recommendations contained in this document were developed and compiled by the Division of Safety, National Institute of Allergy and Infectious Diseases, the National Cancer Institute, and the Clinical Center of the National Institutes of Health; Food and Drug Administration; and the following CDC units: AIDS Program, Hospital Infections Program, Office of the Director, Center for Infectious Diseases; the Training and Laboratory Program Office; and the Office of Biosafety, Office of the Centers Director; Representatives of the following organizations also collaborated in the effort: the American Academy of Microbiology, the American Biological Safety Association, the American Society for Microbiology, the American Society for Clinical Pathology, the Association of State and Territorial Public Health Laboratory Directors, the College of American Pathologists, the Pharmaceutical Manufacturers Association, and the Walter Reed Army Institute for Research.

†Available from Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402, Stock #01702300167-1; or from National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, Stock #PB84-206879.

The HIV agent summary statement does not specifically address safety measures for collecting and handling clinical specimens. Nonetheless, it has been recommended that blood and body-fluid precautions consistently be used for ALL specimens from ALL patients. This approach, referred to as "universal blood and body-fluid precautions" or "universal precautions," eliminates the need to identify all patients infected with HIV (or other bloodborne pathogens) (*3*). This subject is also covered in other publications (*3-8*).

Laboratory directors, supervisors, and others are asked to attach a copy of this revised "1988 Agent Summary Statement for Human Immunodeficiency Virus" to each copy of the *Guidelines* and to all copies of their laboratory biosafety manual; they should review the recommended precautions with laboratory personnel, provide appropriate training in practices and operation of facilities, and ensure that all personnel demonstrate proficiency BEFORE being allowed to work with HIV. The laboratory director (or the designated laboratory supervisor) is responsible for biosafety in the laboratory and must establish and implement practices, facilities, equipment, training, and work assignments as appropriate (*9*).

HIV AGENT SUMMARY STATEMENT

Agent: HIVs Including HTLV-III, LAV, HIV-1, and HIV-2

In the period 1984-1986, several health-care workers (HCWs) who had no recognized risk behavior for acquired immunodeficiency syndrome (AIDS) were reported to have HIV infection (*10-15*). Only one of these HCWs was identified as a laboratory worker. These and other reports assessed the risk of work-related HIV infection for all HCWs as being very low (*3,6,10-12,14-18*).

In 1985, anecdotal reports were received indicating that workers in two different HIV-reagent-production laboratories had been exposed to droplets and splashed liquid from a vessel containing concentrated virus. One of several workers had been cut by glass from a broken carboy that contained HIV-infected cells and medium. None of the persons exposed in these episodes had developed antibody to HIV or had clinical signs of infection 18 and 20 months, respectively, after the reported exposure.

In 1987, CDC received reports that three HCWs had HIV infection; none of the infections were associated with needlesticks or cuts. Two of these HCWs were clinical laboratory workers (*11*). One was a phlebotomist whose face and mouth were splattered with a patient's blood when the rubber stopper was suddenly expelled from a blood-collection tube. The second was a medical technologist who inadvertently spilled blood on her arms and forearms while using an apheresis apparatus to process blood from an HIV-seropositive patient.

In September 1987, a production-laboratory worker was reported to have HIV infection (*18*). This person worked with large concentrations of HIV in a BSL 3 facility. HIV was isolated from the worker's blood; the isolate was genetically indistinguishable from the strain of virus being cultivated in the laboratory. No risk factors were identified, and the worker recalled no specific incident that might have led to infection. However, there were instances of leakage of virus-positive culture fluid from equipment and contamination of the work area and centrifuge rotors. The report

concluded that the most plausible source of exposure was contact of the worker's gloved hand with virus-culture supernatant, followed by inapparent exposure to skin.

In October 1987, a second person who worked in another HIV production facility was reported to have HIV infection (*18*). This laboratory was a well-equipped BSL 3 facility, and BSL 3 practices were being followed. This worker reported having sustained a puncture wound to a finger while cleaning equipment used to concentrate HIV.

Laboratory Hazards

HIV has been isolated from blood, semen, saliva, tears, urine, cerebrospinal fluid, amniotic fluid, breast milk, cervical secretions, and tissue of infected persons and experimentally infected nonhuman primates. In the laboratory, virus should be presumed to be present in all HIV cultures, in all materials derived from HIV cultures, and in/on all equipment and devices coming into direct contact with any of these materials.

In the laboratory, the skin (especially when scratches, cuts, abrasions, dermatitis, or other lesions are present) and mucous membranes of the eye, nose, mouth, and possibly the respiratory tract should be considered as potential pathways for entry of virus. Needles, sharp instruments, broken glass, and other sharp objects must be carefully handled and properly discarded. Care must be taken to avoid spilling and splashing infected cell-culture liquid and other virus-containing materials.

Recommended Precautions

1. BSL 2 standards and special practices, containment equipment, and facilities, as described in the CDC-NIH publication *Biosafety in Microbiological and Biomedical Laboratories* (*Guidelines*), are recommended for activities involving all clinical specimens, body fluids, and tissues from humans or from infected or inoculated laboratory animals. These are the same standards and practices recommended for handling all clinical specimens. For example, and for emphasis:
   a. Use of syringes, needles, and other sharp instruments should be avoided if possible. Used needles and disposable cutting instruments should be discarded into a puncture-resistant container with a lid. Needles should not be re-sheathed, bent, broken, removed from disposable syringes, or otherwise manipulated by hand.
   b. Protective gloves should be worn by all personnel engaged in activities that may involve direct contact of skin with potentially infectious specimens, cultures, or tissues. Gloves should be carefully removed and changed when they are visibly contaminated. Personnel who have dermatitis or other lesions on the hands and who may have indirect contact with potentially infectious material should also wear protective gloves. Hand washing with soap and water immediately after infectious materials are handled and after work is completed—EVEN WHEN GLOVES HAVE BEEN WORN as described above—should be a routine practice.
   c. Generation of aerosols, droplets, splashes, and spills should be avoided. A biological safety cabinet should be used for all procedures that might generate aerosols or droplets and for all infected cell-culture manipulations. The *Guidelines* (pp. 11-13) contain additional precautions for operating at BSL 2.

2. Activities such as producing research-laboratory-scale amounts of HIV, manipulating concentrated virus preparations, and conducting procedures that may produce aerosols or droplets should be performed in a BSL 2 facility with the additional practices and containment equipment recommended for BSL 3 (*19*) (*Guidelines*, pp. 14-17).
3. Activities involving industrial-scale, large-volume production or high concentration and manipulation of concentrated HIV should be conducted in a BSL 3 facility using BSL 3 practices and equipment (*19*).
4. BSL 2 practices, containment equipment, and facilities for animals are recommended for activities involving nonhuman primates and any animals experimentally infected or inoculated with HIV. Because laboratory animals may bite, throw feces or urine, or expectorate at humans, animal-care personnel, investigators, technical staff, and other persons who enter the animal rooms should wear coats, protective gloves, coveralls or uniforms, and—as appropriate—face shields or surgical masks and eye shields to protect the skin and mucous membranes of the eyes, nose, and mouth.
5. All laboratory glassware, disposable material, and waste material suspected or known to contain HIV should be decontaminated, preferably in an autoclave, before it is washed, discarded, etc. An alternate method of disposing of solid wastes is incineration.
6. Laboratory workers should wear laboratory coats, gowns, or uniforms when working with HIV or with material known or suspected to contain HIV. There is no evidence that laboratory clothing poses a risk for HIV transmission; however, clothing that becomes contaminated with HIV preparations should be decontaminated before being laundered or discarded. Laboratory personnel must remove laboratory clothing before going to nonlaboratory areas.
7. Work surfaces should be decontaminated with an appropriate chemical germicide after procedures are completed, when surfaces are overtly contaminated, and at the end of each work day. Many commercially available chemical disinfectants (*5,20-23*) can be used for decontaminating laboratory work surfaces, for some laboratory instruments, for spot cleaning of contaminated laboratory clothing, and for spills of infectious materials. Prompt decontamination of spills should be standard practice.
8. Universal precautions are recommended for handling all human blood specimens for hematologic, microbiologic, chemical, serologic testing; these are the same precautions for preventing transmission of all bloodborne infections including hepatitis B (*17,21,24,25*). It is not certain how effective 56 C-60 C heat is in destroying HIV in serum (*22,23,26*), but heating small volumes of serum for 30 minutes at 56 C before serologic testing reduces residual infectivity to below detectable levels. Such treatment causes some false-positive results in HIV enzyme immunoassays (*27-30*) and may also affect some biochemical assays performed on serum (*27,31,32*).
9. Human serum from any source that is used as a control or reagent in a test procedure should be handled at BSL 2 (*Guidelines*, pp. 11-13). Addendum 2 (p. 16) to this report is a statement issued by CDC on the use of all human control and reagent serum specimens shipped to other laboratories. The Food and Drug Administration requires that manufacturers of human serum reagents use a similarly worded statement.

10. Medical surveillance programs should be in place in all laboratories that test specimens, do research, or produce reagents involving HIV. The nature and scope of a surveillance program will vary according to institutional policy and applicable local, state, and Federal regulations (*9*).
11. If a laboratory worker has a parenteral or mucous-membrane exposure to blood, body fluid, or viral-culture material, the source material should be identified and, if possible, tested for the presence of virus. If the source material is positive for HIV antibody, virus, or antigen, or is not available for examination, the worker should be counseled regarding the risk of infection and should be evaluated clinically and serologically for evidence of HIV infection. The worker should be advised to report on and to seek medical evaluation of any acute febrile illness that occurs within 12 weeks after the exposure (*3*). Such an illness—particularly one characterized by fever, rash, or lymphadenopathy—may indicate recent HIV infection. If seronegative, the worker should be retested 6 weeks after the exposure and periodically thereafter (e.g., at 12 weeks and 6 months after exposure). During this follow-up period—especially during the first 6-12 weeks after exposure, when most infected persons are expected to show serologic evidence of infection—exposed workers should be counseled to follow Public Health Service recommendations for preventing transmission of HIV (*3,14,25,33*). It is recommended that all institutions establish written policies regarding the management of laboratory exposure to HIV; such policies should deal with confidentiality, counseling, and other related issues.
12. Other primary and opportunistic pathogenic agents may be present in the body fluids and tissues of persons infected with HIV. Laboratory workers should follow accepted biosafety practices to ensure maximum protection against inadvertent laboratory exposure to agents that may also be present in clinical specimens (*34-36*).
13. Unless otherwise dictated by institutional policy, the laboratory director (or designated laboratory supervisor) is responsible for carrying out the biosafety program in the laboratory. In this regard, the laboratory director or designated supervisor should establish the biosafety level for each component of the work to be done and should ensure that facilities and equipment are adequate and in good working order, that appropriate initial and periodic training is provided to the laboratory staff, and that recommended practices and procedures are strictly followed (*9*).
14. Attention is directed to a "Joint Advisory Notice" of the Departments of Labor and Health and Human Services (*9*) that describes the responsibility of employers to provide "safe and healthful working conditions" to protect employees against occupational infection with HIV. The notice defines three exposure categories of generic job-related tasks and describes the protective measures required for employees involved in each exposure category. These measures are: administrative measures, training and education programs for employees, engineering controls, work practices, medical and health-care practices, and record-keeping. The recommendations in this report are consistent with the "Joint Advisory Notice"; managers/directors of all biomedical laboratories are urged to read this notice.

ADDENDUM 1

LABORATORY BIOSAFETY LEVEL CRITERIA

Biosafety Level 2

Biosafety Level 2 is similar to Level 1 and is suitable for work involving agents that represent a moderate hazard for personnel and the environment. It differs in that a) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists, b) access to the laboratory is limited when work is being conducted, and c) certain procedures in which infectious aerosols are created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

A. Standard microbiological practices
   1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress.
   2. Work surfaces are decontaminated at least once a day and after any spill of viable material.
   3. All infectious liquid or solid waste is decontaminated before being disposed of.
   4. Mechanical pipetting devices are used; mouth pipetting is prohibited.
   5. Eating, drinking, smoking, and applying cosmetics are not permitted in the work area. Food must be stored in cabinets or refrigerators designed and used for this purpose only. Food storage cabinets or refrigerators should be located outside the work area.
   6. Persons are to wash their hands when they leave the laboratory after handling infectious material or animals.
   7. All procedures are performed carefully to minimize the creation of aerosols.

B. Special practices
   1. Contaminated materials that are to be decontaminated away from the laboratory are placed in a durable, leakproof container that is closed before being removed from the laboratory.
   2. The laboratory director limits access to the laboratory. In general, persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
   3. The laboratory director establishes policies or procedures whereby only persons who have been advised of the potential hazard and who meet any specific entry requirements (e.g., vaccination) enter the laboratory or animal rooms.
   4. When an infectious agent being worked with in the laboratory requires special provisions for entry (e.g., vaccination), a hazard warning sign that incorporates the universal biohazard symbol is posted on the access door to the laboratory work area. The hazard warning sign identifies the infec-

tious agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates the special requirement(s) for entering the laboratory.

5. An insect and rodent control program is in effect.
6. Laboratory coats, gowns, smocks, or uniforms are worn while in the laboratory. Before leaving the laboratory for nonlaboratory areas (e.g., cafeteria, library, administrative offices), this protective clothing is removed and left in the laboratory or covered with a clean coat not used in the laboratory.
7. Animals not involved in the work being performed are not permitted in the laboratory.
8. Special care is taken to avoid having skin be contaminated with infectious material; gloves should be worn when handling infected animals and when skin contact with infectious material is unavoidable.
9. All waste from laboratories and animal rooms is appropriately decontaminated before disposal.
10. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of infectious fluid. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before discard or reuse.
11. Spills and accidents that result in overt exposures to infectious material are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained.
12. When appropriate, considering the agent(s) handled, baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or on the function of the facility.
13. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

C. Containment equipment

Biological safety cabinets (Class I or II) or other appropriate personal-protection or physical-containment devices are used when:

1. Procedures with a high potential for creating infectious aerosols are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or eggs.
2. High concentrations or large volumes of infectious agents are used. Some types of materials may be centrifuged in the open laboratory if sealed heads

or centrifuge safety cups are used and if the containers are opened only in a biological safety cabinet.

D. Laboratory facilities
1. The laboratory is designed so that it can be easily cleaned.
2. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
3. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
4. Each laboratory contains a sink for hand washing.
5. If the laboratory has windows that open, they are fitted with fly screens.
6. An autoclave for decontaminating infectious laboratory wastes is available.

Biosafety Level 3

Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by inhalation. Laboratory personnel have specific training in handling pathogenic and/or potentially lethal agents and are supervised by competent scientists who are experienced in working with these agents. All procedures involving the manipulation of infectious material are conducted within biological safety cabinets or other physical containment devices or by personnel wearing appropriate personal-protection clothing and devices. The laboratory has special engineering and design features. It is recognized, however, that many existing facilities may not have all the facility safeguards recommended for Biosafety Level 3 (e.g., access zone, sealed penetrations, and directional airflow). In these circumstances, acceptable safety may be achieved for routine or repetitive operations (e.g., diagnostic procedures involving the propagation of an agent for identification, typing, and susceptibility testing) in laboratories in which facility features satisfy Biosafety Level 2 recommendations if the recommended "Standard Microbiological Practices," "Special Practices," and "Containment Equipment" for Biosafety Level 3 are rigorously followed. The decision to implement this modification of Biosafety Level 3 recommendations should be made only by the laboratory director.

The following standard and special safety practices, equipment, and facilities apply to agents assigned to Biosafety Level 3:

A. Standard microbiological practices
1. Work surfaces are decontaminated at least once a day and after any spill of viable material.
2. All infectious liquid or solid waste is decontaminated before being disposed of.
3. Mechanical pipetting devices are used; mouth pipetting is prohibited.
4. Eating, drinking, smoking, storing food, and applying cosmetics are not permitted in the work area.
5. Persons wash their hands after handling infectious materials and animals and every time they leave the laboratory.
6. All procedures are performed carefully to minimize the creation of aerosols.

B. Special practices
1. Laboratory doors are kept closed when experiments are in progress.

2. Contaminated materials that are to be decontaminated at a site away from the laboratory are placed in a durable, leakproof container that is closed before being removed from the laboratory.
3. The laboratory director controls access to the laboratory and limits access only to persons whose presence is required for program or support purposes. Persons who are at increased risk of acquiring infection or for whom infection may be unusually hazardous are not allowed in the laboratory or animal rooms. The director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory.
4. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements (e.g., vaccination), and who comply with all entry and exit procedures enter the laboratory or animal rooms.
5. When infectious materials or infected animals are present in the laboratory or containment module, a hazard warning sign (incorporating the universal biohazard symbol) is posted on all laboratory and animal-room access doors. The hazard warning sign identifies the agent, lists the name and telephone number of the laboratory director or other responsible person(s), and indicates any special requirements for entering the laboratory, such as the need for vaccinations, respirators, or other personal-protection measures.
6. All activities involving infectious materials are conducted in biological safety cabinets or other physical-containment devices within the containment module. No work is conducted in open vessels on the open bench.
7. The work surfaces of biological safety cabinets and other containment equipment are decontaminated when work with infectious materials is finished. Plastic-backed paper toweling used on nonperforated work surfaces within biological safety cabinets facilitates clean-up.
8. An insect and rodent control program is in effect.
9. Laboratory clothing that protects street clothing (e.g., solid-front or wrap-around gowns, scrub suits, coveralls) is worn in the laboratory. Laboratory clothing is not worn outside the laboratory, and it is decontaminated before being laundered.
10. Special care is taken to avoid skin contamination with infectious materials; gloves are worn when handling infected animals and when skin contact with infectious materials is unavoidable.
11. Molded surgical masks or respirators are worn in rooms containing infected animals.
12. Animals and plants not related to the work being conducted are not permitted in the laboratory.
13. All waste from laboratories and animal rooms is appropriately decontaminated before being disposed of.
14. Vacuum lines are protected with high-efficiency particulate air (HEPA) filters and liquid disinfectant traps.
15. Hypodermic needles and syringes are used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle

is integral to the syringe) are used for the injection or aspiration of infectious fluids. Extreme caution is used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused.

16. Spills and accidents that result in overt or potential exposures to infectious material are immediately reported to the laboratory director. Appropriate medical evaluation, surveillance, and treatment are provided, and written records are maintained.
17. Baseline serum samples for all laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the laboratory.
18. A biosafety manual is prepared or adopted. Personnel are advised of special hazards and are required to read instructions on practices and procedures and to follow them.

C. Containment equipment

Biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal-protection or physical-containment devices (e.g., special protective clothing, masks, gloves, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals) are used for all activities with infectious materials that pose a threat of aerosol exposure. These include: manipulation of cultures and of clinical or environmental material that may be a source of infectious aerosols; the aerosol challenge of experimental animals; harvesting of tissues or fluids from infected animals and embryonated eggs; and necropsy of infected animals.

D. Laboratory facilities

1. The laboratory is separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors is the basic requirement for entry into the laboratory from access corridors or other contiguous areas. Physical separation of the high-containment laboratory from access corridors or other laboratories or activities may also be provided by a double-doored clothes-change room (showers may be included), airlock, or other access facility that requires passing through two sets of doors before entering the laboratory.
2. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be easily cleaned. Penetrations in these surfaces are sealed or capable of being sealed to facilitate decontaminating the area.
3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.
4. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
5. Each laboratory contains a sink for washing hands. The sink is foot, elbow, or automatically operated and is located near the laboratory exit door.
6. Windows in the laboratory are closed and sealed.
7. Access doors to the laboratory or containment module are self-closing.

8. An autoclave for decontaminating laboratory wastes is available, preferably within the laboratory.
9. A ducted exhaust-air ventilation system is provided. This system creates directional airflow that draws air into the laboratory through the entry area. The exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow is proper (i.e., into the laboratory). The exhaust air from the laboratory room can be discharged to the outside without being filtered or otherwise treated.
10. The HEPA-filtered exhaust air from Class I or CLass II biological safety cabinets is discharged directly to the outside or through the building exhaust system. Exhaust air from Class I or II biological safety cabinets may be recirculated within the laboratory if the cabinet is tested and certified at least every 12 months. If the HEPA-filtered exhaust air from Class I or II biological safety cabinets is to be discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble-unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

VERTEBRATE ANIMAL BIOSAFETY LEVEL CRITERIA

Animal Biosafety Level 2

A. Standard practices

1. Doors to animal rooms open inward, are self-closing, and are kept closed when infected animals are present.
2. Work surfaces are decontaminated after use or spills of viable materials.
3. Eating, drinking, smoking, and storing of food for human use are not permitted in animal rooms.
4. Personnel wash their hands after handling cultures and animals and before leaving the animal room.
5. All procedures are carefully performed to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

B. Special practices

1. Cages are decontaminated, preferably by autoclaving, before being cleaned and washed.
2. Surgical-type masks are worn by all personnel entering animal rooms housing nonhuman primates.
3. Laboratory coats, gowns, or uniforms are worn while in the animal room. This protective clothing is removed before leaving the animal facility.
4. The laboratory or animal-facility director limits access to the animal room only to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when work is in progress. In general, persons who may be at increased risk of acquiring

infection or for whom infection might be unusually hazardous are not allowed in the animal room.

5. The laboratory or animal-facility director establishes policies and procedures whereby only persons who have been advised of the potential hazard and who meet any specific requirements (e.g., vaccination) may enter the animal room.
6. When an infectious agent in use in the animal room requires special-entry provisions (e.g., vaccination), a hazard warning sign (incorporating the universal biohazard symbol) is posted on the access door to the animal room. The hazard warning sign identifies the infectious agent, lists the name and telephone number of the animal-facility supervisor or other responsible person(s), and indicates the special requirement(s) for entering the animal room.
7. Special care is taken to avoid contaminating skin with infectious material; gloves should be worn when handling infected animals and when skin contact with infectious materials is unavoidable.
8. All waste from the animal room is appropriately decontaminated—preferably by autoclaving—before being disposed of. Infected animal carcasses are incinerated after being transported from the animal room in leakproof, covered containers.
9. Hypodermic needles and syringes are used only for the parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) are used for the injection or aspiration of infectious fluids. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused.
10. If floor drains are provided, the drain taps are always filled with water or a suitable disinfectant.
11. When appropriate, considering the agents handled, baseline serum samples from animal-care and other at-risk personnel are collected and stored. Additional serum samples may be collected periodically, depending on the agents handled or the function of the facility.

C. Containment equipment

Biological safety cabinets, other physical-containment devices, and/or personal-protection devices (e.g., respirators, face shields) are used when procedures with a high potential for creating aerosols are conducted. These include necropsy of infected animals, harvesting of infected tissues or fluids from animals or eggs, intranasal inoculation of animals, and manipulation of high concentrations or large volumes of infectious materials.

D. Animal facilities

1. The animal facility is designed and constructed to facilitate cleaning and housekeeping.
2. A sink for washing hands is available in the room that houses infected animals.
3. If the animal facility has windows that open, they are fitted with fly screens.

4. It is recommended, but not required, that the direction of airflow in the animal facility is inward and that exhaust air is discharged to the outside without being recirculated to other rooms.
5. An autoclave that can be used for decontaminating infectious laboratory waste is available in the same building that contains the animal facility.

Animal Biosafety Level 3

A. Standard practices

1. Doors to animal rooms open inward, are self-closing, and are kept closed when work with infected animals is in progress.
2. Work surfaces are decontaminated after use or after spills of viable materials.
3. Eating, drinking, smoking, and storing of food for human use are not permitted in the animal room.
4. Personnel wash their hands after handling cultures or animals and before leaving the laboratory.
5. All procedures are carefully performed to minimize the creation of aerosols.
6. An insect and rodent control program is in effect.

B. Special practices

1. Cages are autoclaved before bedding is removed and before they are cleaned and washed.
2. Surgical-type masks or other respiratory protection devices (e.g., respirators) are worn by personnel entering rooms that house animals infected with agents assigned to Biosafety Level 3.
3. Wrap-around or solid-front gowns or uniforms are worn by personnel entering the animal room. Front-button laboratory coats are unsuitable. Protective gowns must remain in the animal room and must be decontaminated before being laundered.
4. The laboratory director or other responsible person limits access to the animal room only to personnel who have been advised of the potential hazard and who need to enter the room for program or service purposes when infected animals are present. In general, persons who may be at increased risk of acquiring infection or for whom infection might be unusually hazardous are not allowed in the animal room.
5. The laboratory director or other responsible person establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet any specific requirements (e.g., vaccination) may enter the animal room.
6. Hazard warning signs (incorporating the universal biohazard warning symbol) are posted on access doors to animal rooms containing animals infected with agents assigned to Biosafety Level 3 are present. The hazard warning sign should identify the agent(s) in use, list the name and telephone number of the animal room supervisor or other responsible person(s), and indicate any special conditions of entry into the animal room (e.g., the need for vaccinations or respirators).
7. Personnel wear gloves when handling infected animals. Gloves are removed aseptically and autoclaved with other animal room waste before being disposed of or reused.

8. All wastes from the animal room are autoclaved before being disposed of. All animal carcasses are incinerated. Dead animals are transported from the animal room to the incinerator in leakproof, covered containers.
9. Hypodermic needles and syringes are used only for gavage or parenteral injection or aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) are used. A needle should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe should be promptly placed in a puncture-resistant container and decontaminated, preferably by autoclaving, before being discarded or reused. When possible, cannulas should be used instead of sharp needles (e.g., gavage).
10. If floor drains are provided, the drain traps are always filled with water or a suitable disinfectant.
11. If vacuum lines are provided, they are protected with HEPA filters and liquid disinfectant traps.
12. Boots, shoe covers, or other protective footwear and disinfectant footbaths are available and used when indicated.

C. Containment equipment
1. Personal-protection clothing and equipment and/or other physical-containment devices are used for all procedures and manipulations of infectious materials or infected animals.
2. The risk of infectious aerosols from infected animals or their bedding can be reduced if animals are housed in partial-containment caging systems, such as open cages placed in ventilated enclosures (e.g., laminar-flow cabinets), solid-wall and -bottom cages covered by filter bonnets, or other equivalent primary containment systems.

D. Animal facilities
1. The animal facility is designed and constructed to facilitate cleaning and housekeeping and is separated from areas that are open to unrestricted personnel traffic within the building. Passage through two sets of doors is the basic requirement for entry into the animal room from access corridors or other contiguous areas. Physical separation of the animal room from access corridors or from other activities may also be provided by a double-doored clothes change room (showers may be included), airlock, or other access facility that requires passage through two sets of doors before entering the animal room.
2. The interior surfaces of walls, floors, and ceilings are water resistant so that they can be cleaned easily. Penetrations in these surfaces are sealed or capable of being sealed to facilitate fumigation or space decontamination.
3. A foot, elbow, or automatically operated sink for hand washing is provided near each animal-room exit door.
4. Windows in the animal room are closed and sealed.
5. Animal room doors are self-closing and are kept closed when infected animals are present.
6. An autoclave for decontaminating wastes is available, preferably within the animal room. Materials to be autoclaved outside the animal room are transported in a covered, leakproof container.

7. An exhaust-air ventilation system is provided. This system creates directional airflow that draws air into the animal room through the entry area. The building exhaust can be used for this purpose if the exhaust air is not recirculated to any other area of the building, is discharged to the outside, and is dispersed away from occupied areas and air intakes. Personnel must verify that the direction of the airflow is proper (i.e., into the animal room). The exhaust air from the animal room that does not pass through biological safety cabinets or other primary containment equipment can be discharged to the outside without being filtered or otherwise treated.
8. The HEPA-filtered exhaust air from Class I or Class II biological safety cabinets or other primary containment devices is discharged directly to the outside or through the building's exhaust system. Exhaust air from these primary containment devices may be recirculated within the animal room if the cabinet is tested and certified at least every 12 months. If the HEPA-filtered exhaust air from Class I or Class II biological safety cabinets is discharged to the outside through the building exhaust system, it is connected to this system in a manner (e.g., thimble-unit connection) that avoids any interference with the air balance of the cabinets or building exhaust system.

ADDENDUM 2

CDC cautionary notice for all human-serum-derived reagents used as controls:

WARNING: Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, this specimen should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories*, 1984, pages 11-13.

If additional statements describing the results of any heat treatment or serologic procedure(s) already performed on the human-serum reagent or control are used in conjunction with the above cautionary notice, these statements should be worded so as not to diminish the impact of the warning that emphasizes the need for universal precautions.

References

1. Richardson JH, Barkley WE, eds. Biosafety in microbiological and biomedical laboratories, 1984. Washington, DC: Public Health Service, 1984; DHHS publication no. (CDC)84-8395.
2. CDC. Human T-lymphotropic virus type III/lymphadenopathy-associated virus agent summary statement. MMWR 1986;35:540-2, 547-9.
3. CDC. Recommendations for prevention of HIV transmission in health-care settings. MMWR 1987;36(suppl 2):3S-18S.
4. Isenberg HD, Washington JA, Balows A, Sonnenwirth AC. Collection, handling and processing of specimens. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ, eds. Manual of clinical microbiology, 4th ed. Washington, DC: American Society for Microbiology, 1985:73-98.
5. CDC. Acquired immune deficiency syndrome (AIDS): precautions for clinical and laboratory workers. MMWR 1982;32:577-80.
6. CDC. Recommendations for preventing transmission of infection with human T-lymphotropic virus type III/lymphadenopathy-associated virus in the workplace. MMWR 1985;34:681.
7. CDC. Recommendations for preventing transmission of infection with human T-lymphotropic virus type III/lymphadenopathy-associated virus during invasive procedures. MMWR 1986;35:221-3.
8. CDC. Recommendations for preventing transmission of infection with human T-lymphotropic virus type III/lymphadenopathy-associated virus in the workplace. MMWR 1985;34:682-6, 691-5.
9. U.S. Department of Health and Human Services, Public Health Service. Joint advisory notice: HBV/HIV. Federal Register 1987;52 (October 30): 41818-24.
10. CDC. Update: evaluation of human T-lymphotropic virus type III/lymphadenopathy-associated virus infection in health-care personnel—United States. MMWR 1985;34:575-8.
11. CDC. Human immunodeficiency virus infections in health-care workers exposed to blood of infected patients. MMWR 1987;36:285-9.
12. Anonymous. Needlestick transmission of HTLV-III from a patient infected in Africa. Lancet 1984;2:1376-7.
13. Stricof RL, Morse DL. HTLV-III/LAV seroconversion following a deep intramuscular needlestick injury. New Engl J Med 1986;314:1115.
14. McCray E, Cooperative Needlestick Study Group. Occupational risk of the acquired immunodeficiency syndrome among health-care workers. N Engl J Med 1986;314:1127-32.
15. Weiss SH, Saxinger WC, Richtman D, et al. HTLV-III infection among health-care workers: association with needlestick injuries. JAMA 1985;254:2089-93.

16. Henderson DK, Saah AJ, Zak BJ, et al. Risk of nosocomial infection with human T-cell lymphotropic virus type III/lymphadenopathy-associated virus in a large cohort of intensively exposed health care workers. Ann Intern Med 1986;104:644-7.
17. Gerberding JL, Bryant-Le Blanc CE, Nelson K, et al. Risk of transmitting the human immunodeficiency virus, cytomegalovirus, and hepatitis B virus to health-care workers exposed to patients with AIDS and AIDS-related conditions. J Infect Dis 1987;156:1-8.
18. Weiss SH, Goedert JJ, Gartner S, et al. Risk of human immunodeficiency virus (HIV-1) infection among laboratory workers. Science 1988;239:68-71.
19. U.S. Department of Health and Human Services, Public Health Service. Biosafety guidelines for use of HTLV-III and related viruses. Federal Register 1984 (October 16);49:40556.
20. U.S. Environmental Protection Agency. EPA guide for infectious waste management. Washington, DC: U.S. Environmental Protection Agency, 1986; Publication no. EPA/530-SW-86-014.
21. Favero MS. Sterilization, disinfection and antisepsis in the hospital. In: Lennette EH, Balows A, Hausler WJ, Shadomy HJ, eds. Manual of clinical microbiology, 4th ed. Washington, DC: American Society for Microbiology: 1985;129-37.
22. Martin LS, McDougal JS, Loskoski SL. Disinfection and inactivation of the human T lymphotropic virus type III/lymphadenopathy-associated virus. J Infect Dis 1985:152:400-3.
23. Resnick L, Veren K, Salahuddin, SZ, Tondreau S, Markham PD. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. JAMA 1986;255:1887-91.
24. Favero MS, Petersen NJ, Bond WW. Transmission and control of laboratory-acquired hepatitis infection. In: Miller BM, Groschel DHM, Richardson JH, et al., eds. Laboratory safety: principles and practice. Washington, DC: American Society for Microbiology, 1986:49-58.
25. CDC. Public Health Service guidelines for counseling and antibody testing to prevent HIV infections and AIDS. MMWR 1987;36:509-15.
26. Ronalds CJ, Grint PCA, Kangro HD. Disinfection and inactivation of HTLV-III/LAV [Letter]. J Infect Dis 1986;153:996.
27. Evans RP, Shanson DC. Effect of heat on serologic tests for hepatitis B and syphilis and on aminoglycoside assays [Letter]. Lancet 1985;1:1458.
28. Van den Akker R, Hekker AC, Osterhaus ADME. Heat inactivation of serum may interfere with HTLV-III/LAV serology [Letter]. Lancet 1985;2:672.
29. Mortimer PP, Parry JV, Mortimer JY. Which anti-HTLV III/LAV assays for screening and confirmatory testing? Lancet 1985:2:873-7.
30. Jungkind DL, DiRenzo SA, Young SJ. Effect of using heat-inactivated serum with the Abbott human T-cell lymphotropic virus type III antibody test. J Clin Microbiol 1986;23:381-2.
31. Goldie DJ, McConnell AA, Cooke PR. Heat treatment of whole blood and serum before chemical analysis [Letter]. Lancet 1985;1:1161.
32. Lai L, Ball G, Stevens J, Shanson D. Effect of heat treatment of plasma and serum on biochemical indices [Letter]. Lancet 1985;1:1457-8.
33. CDC. Additional recommendations to reduce sexual and drug abuse-related transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus. MMWR 1986;35:152-5.
34. CDC. Revision of the case definition of acquired immunodeficiency syndrome for national reporting—United States. MMWR 1985;34:373-5.
35. CDC. Diagnosis and management of mycobacterial infection and disease in persons with human T-lymphotropic virus type III/lymphadenopathy-associated virus infection. MMWR 1986;35:448-52.
36. CDC. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. MMWR 1987;36(suppl 1):1S-15S.

Occupationally Acquired Human Immunodeficiency Virus Infections in Laboratories Producing Virus Concentrates in Large Quantities:

Conclusions and Recommendations of an Expert Team Convened by the Director of the National Institutes of Health (NIH)

*Reported by Division of Safety, National Institutes of Health**

INTRODUCTION

The recommendations of the expert team are directed to industrial-scale facilities for the production of large quantities of highly concentrated HIV. Their recommendations are similar to and complement those in the preceding "1988 Agent Summary Statement for Human Immunodeficiency Virus," which updates the one published in 1986 (*1*). Laboratory directors and others responsible for the health and safety of laboratory employees working with HIV and HIV-containing material should carefully consider these relevant recommendations and guidelines in developing an appropriate safety program.

COMMITTEE REPORT

Two workers in different laboratories producing large quantities of highly concentrated HIV have been reported to have laboratory-acquired HIV infections (*1*). One worker's infection was presumed to be caused by "undetected skin contact with virus culture supernatant" (*2*). The other worker's infection followed "an injury with a potentially contaminated needle" (*2*). After the first case was identified, the Director of NIH convened a team of experts to investigate the incidents and to visit seven different laboratories that produced large volumes of HIV. After facilities inspections and separate, confidential interviews with the workers, the team prepared a report of their findings. The conclusions and recommendations from that report follow.

*Expert Team: W. Emmett Barkley, PhD, Director, Division of Engineering Services, National Institutes of Health; Robert McKinney, PhD, Director, Division of Safety, National Institutes of Health; John Richardson, DVM, MPH, Biosafety Officer, Emory University; Gerald Schochetman, PhD, Chief, Laboratory Investigations Branch, AIDS Program, Center for Infectious Diseases, Centers for Disease Control; David Henderson, MD, Hospital Epidemiologist, Warren Grant Magnuson Clinical Center, National Institutes of Health.

The most probable cause for the first laboratory-acquired infection was inapparent parenteral exposure. Frequent opportunities for unrecognized direct contact with contaminated materials and surfaces were reported to be present. Gloves of questionable integrity, skin cuts and abrasions, and one episode of a dermatitis-like condition represented portals for possible exposure and routes of infection. The inexperience of the first infected worker in microbiologic procedures and Biosafety Level (BSL) 3 practices, coupled with the reliance on obtaining necessary skills through on-the-job training in a setting in which episodes of contamination may have occurred frequently, suggests that the worker might not have possessed an appropriate level of proficiency when the infection may have occurred.

The most probable cause for the second worker's infection was parenteral inoculation. This worker recalled incurring an injury with a blunt cannula approximately 6 months before the first seropositive sample. Incidents of contamination, such as those reported by the first worker, occurred infrequently in the second worker's laboratory.

Aerosol transmission is considered to be the least likely cause of infection in both cases. Operations in which aerosols may have been generated were carried out in biological safety cabinets to reduce the potential for inhalation exposure. Although some aerosols may have been released during the few reported rotor-seal failures involving the continuous-flow zonal centrifuge, the potential for contact exposure was greater. Aerosol transmission was unlikely because: a) in situations in which overt aerosol exposure has occurred in laboratory and production operations involving HIV, no exposed workers have seroconverted; b) no evidence exists that suggests aerosols may be a natural mode of HIV transmission; c) the probable cause identified above is consistent with documented modes of transmission of bloodborne pathogens in the laboratory.

The occurrence of these two infections emphasizes the finite risk that exists for laboratory workers who handle concentrated preparations of HIV. The conclusions of a National Cancer Institute prospective cohort study (*2*) indicate that this risk is low and may be similar to the risk for infection of health-care workers who have experienced a needlestick injury.

The occupational risk for infection by parenteral exposure is substantially reduced or eliminated by strict adherence to BSL 2 practices. The recommended use of BSL 3 practices for highly concentrated preparations of HIV is appropriate. The review of these two infected laboratory workers does not suggest the need to alter current CDC/NIH biosafety recommendations for HIV or for patient care (*3*), research (*1*), or virus production. There is a need, however, for more proficiency and discipline in laboratory safety practices.

The following recommendations will help assure maintenance of a safe and healthy environment for laboratory and production-facility workers who handle concentrated preparations of HIV:

A. Strictly adhere to standard microbiologic practices and techniques

The most important recommendation is to adhere strictly to standard microbiologic practices and techniques. Persons working with HIV must be aware of potential hazards and must be trained and proficient in practice and techniques necessary for self-protection. Employees must be informed that parenteral exposure is the most serious potential hazard for causing a laboratory-acquired infection. They must be able to recognize how such

exposures occur and how they can be prevented. Although on-the-job training is an acceptable approach for learning techniques and practices, it is imperative that proficiency be obtained BEFORE virus is actually handled.

B. Assure that workers are proficient in virus-handling techniques

Selection criteria for employees who will work in production operations or with concentrated preparations of HIV should require experience in the handling of human pathogens or tissue cultures. If an employee has not had such experience, s/he should participate in carefully structured, well-supervised on-the-job training programs.

The director or person in charge of the laboratory or production facility must ensure that personnel are appropriately trained and are proficient in practices and techniques necessary for self-protection. Initial work activities should not include the handling of virus. A progression of work activities should be assigned as techniques are learned and proficiency is developed. Virus should only be introduced into the work activities after the supervisor is confident it can be handled safely.

C. Monitor work practices

Periodically, the biosafety officer or a person with expertise in biosafety should closely observe practices and techniques used in handling HIV. This can be helpful in identifying activities or behavior that may increase the potential for contact with contaminated material or for inapparent parenteral exposures. If deficiencies are noticed, corrective measures should be specified and implemented.

D. Continuously reinforce safe practices

Practices that reduce the potential for direct contact and inapparent parenteral exposure should be continuously reinforced:

- Gloves should always be worn when concentrated preparations of HIV are handled and when contact with a contaminated surface or material may be unavoidable. If a gloved hand accidentally touches a contaminated surface or material, the glove should be removed immediately and the hands washed.
- Work surfaces should be decontaminated at the end of each day and any time contamination is recognized.
- Workers must develop the habit of keeping hands away from the eyes, nose, and mouth in order to avoid potential exposure of mucous membranes. Wearing filter masks and eye goggles or face shields may assist in accomplishing this objective.
- Needles and sharp implements must not be used when HIV is handled unless no acceptable alternative is available. When possible, unbreakable containers should be substituted for glassware, in order to avoid accidental cuts from broken pieces.
- In the absence of advice and consent of an occupational physician or nurse, no worker should handle any virus-containing material when s/he has cuts or skin abrasions on the hands or wrists.

E. Establish a medical surveillance serology program

Each medical facility should have a medical-surveillance serology program. Serum samples should be obtained at least once a year and analyzed for

seroconversion. Results should be reported to individual workers in a timely manner. Counseling services should be available for workers who have positive serologic results. Procedures that maintain strict confidentiality should be adopted.

F. Revalidate integrity of process, transport, and containment equipment

The operational integrity of all equipment used to process, transport, and contain fluids containing HIV should be revalidated at least once a year. The integrity of such equipment should be revalidated after any system failure that releases contaminated fluids into the work environment.

G. Develop production processes that enhance biosafety

Efforts should be made to explore and use production systems and strategies that reduce operational complexity and manual manipulations.

H. Validate efficacy of decontamination methods

Special attention should be given to demonstrating the adequacy of decontamination methods when high organic content, such as cellular debris, is present.

I. Sponsor and conduct biosafety training initiatives

Responsible institutions should orient such programs toward the application of biosafety practices to work involving HIV. Presentation strategies and materials to make the training widely available should be encouraged.

References

1. CDC. Human T-lymphotropic virus type III/lymphadenopathy-associated virus: agent summary statement. MMWR 1986;35:540-2, 547-9.
2. Weiss SH, Goedert JJ, Gartner S, et al. Risk of human immunodeficiency virus (HIV-1) infection among laboratory workers. Science 1988;239:68-71.
3. CDC. Recommendations for prevention of HIV transmission in health-care settings. MMWR 1987;36(suppl 2S):3S-18S.

Supplements to the *MMWR* are published by the Epidemiology Program Office, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia 30333.

SUGGESTED CITATION

Centers for Disease Control. 1988 Agent summary statement for human immunodeficiency virus and Report on laboratory-acquired infection with human immunodeficiency virus. *MMWR* 1988;37(suppl. no. S-4):[inclusive page numbers].

case definition. For reporting purposes, the revision adds to the definition most of those severe non-infectious, non-cancerous HIV-associated conditions that are categorized in the CDC clinical classification systems for HIV infection among adults and children (*4,5*).

Another limitation of the old definition was that AIDS-indicative diseases are diagnosed presumptively (i.e., without confirmation by methods required by the old definition) in 10%-15% of patients diagnosed with such diseases; thus, an appreciable proportion of AIDS cases were missed for reporting purposes (*6,7*). This proportion may be increasing, which would compromise the old case definition's usefulness as a tool for monitoring trends. The revised case definition permits the reporting of these clinically diagnosed cases as long as there is laboratory evidence of HIV infection.

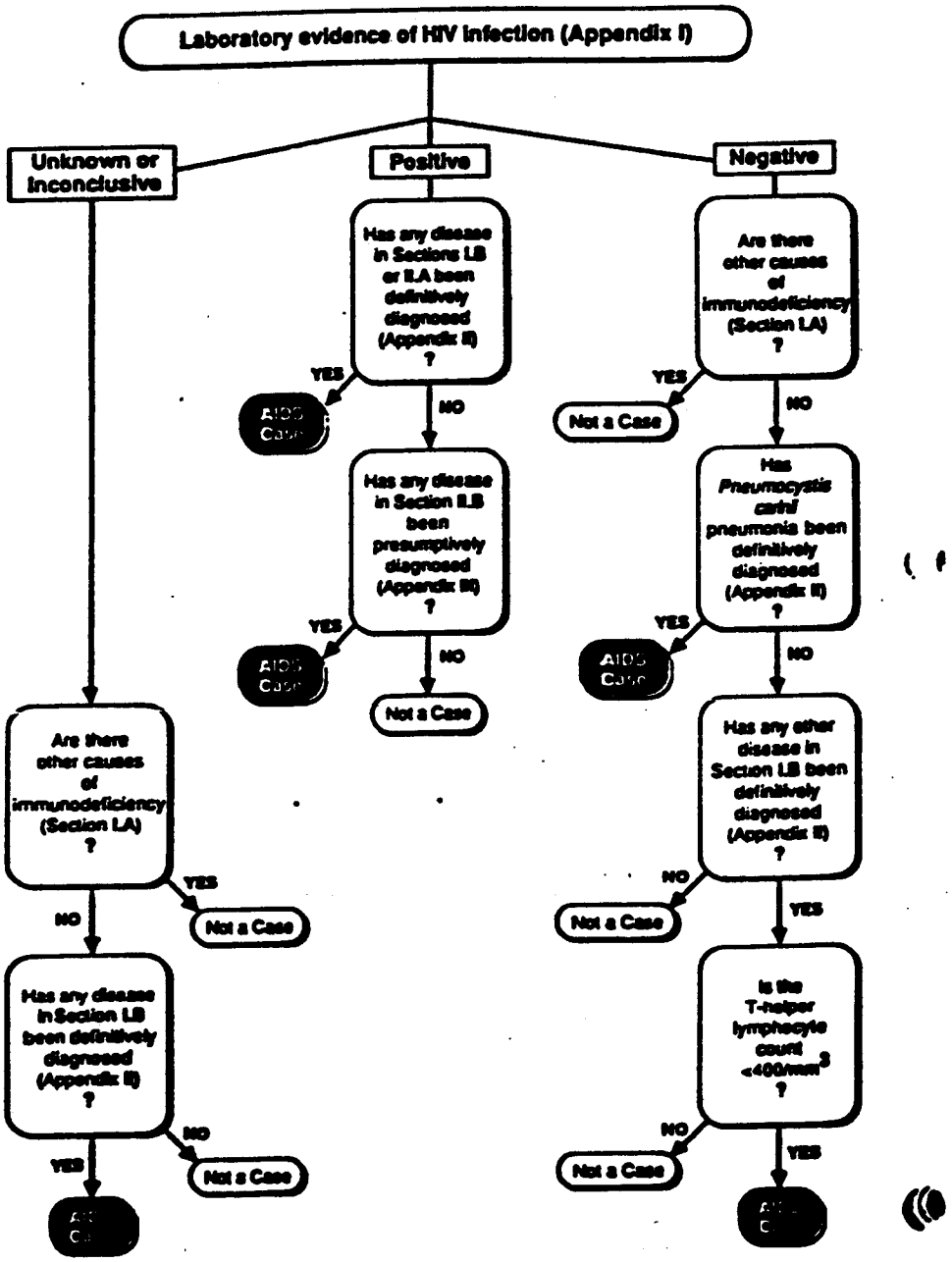
The effectiveness of the revision will depend on how extensively HIV-antibody tests are used. Approximately one third of AIDS patients in the United States have been from New York City and San Francisco, where, since 1985, < 7% have been reported with HIV-antibody test results, compared with > 60% in other areas. The impact of the revision on the reported numbers of AIDS cases will also depend on the proportion of AIDS patients in whom indicator diseases are diagnosed presumptively rather than definitively. The use of presumptive diagnostic criteria varies geographically, being more common in certain rural areas and in urban areas with many indigent AIDS patients.

To avoid confusion about what should be reported to health departments, the term "AIDS" should refer only to conditions meeting the surveillance definition. This definition is intended only to provide consistent statistical data for public health purposes. Clinicians will not rely on this definition alone to diagnose serious disease caused by HIV infection in individual patients because there may be additional information that would lead to a more accurate diagnosis. For example, patients who are not reportable under the definition because they have either a negative HIV-antibody test or, in the presence of HIV antibody, an opportunistic disease not listed in the definition as an indicator of AIDS nonetheless may be diagnosed as having serious HIV disease on consideration of other clinical or laboratory characteristics of HIV infection or a history of exposure to HIV.

Conversely, the AIDS surveillance definition may rarely misclassify other patients as having serious HIV disease if they have no HIV-antibody test but have an AIDS-indicative disease with a background incidence unrelated to HIV infection, such as cryptococcal meningitis.

The diagnostic criteria accepted by the AIDS surveillance case definition should not be interpreted as the standard of good medical practice. Presumptive diagnoses are accepted in the definition because not to count them would be to ignore substantial morbidity resulting from HIV infection. Likewise, the definition accepts a reactive screening test for HIV antibody without confirmation by a supplemental test because a repeatedly reactive screening test result, in combination with an indicator disease, is highly indicative of true HIV disease. For national surveillance purposes, the tiny proportion of possibly false-positive screening tests in persons with AIDS-dicative diseases is of little consequence. For the individual patient, however, a correct diagnosis is critically important. The use of supplemental tests is, therefore, strongly endorsed. An increase in the diagnostic use of HIV-antibody tests could improve both the quality of medical care and the function of the new case definition, as well as assist in providing counselling to prevent transmission of HIV.

FIGURE I. Flow diagram for revised CDC case definition of AIDS, September 1, 1987



References

1. World Health Organization. Acquired immunodeficiency syndrome (AIDS): WHO/CDC case definition for AIDS. WHO Wkly Epidemiol Rec 1986;61:69-72.
2. Haverkos HW, Gottlieb MS, Killen JY, Edelman R. Classification of HTLV-III/LAV-related diseases [Letter]. J Infect Dis 1985;152:1095.
3. Redfield RR, Wright DC, Tramont EC. The Walter Reed staging classification of HTLV-III infection. N Engl J Med 1986;314:131-2.
4. CDC. Classification system for human T-lymphotropic virus type III/lymphadenopathy-associated virus infections. MMWR 1986;35:334-9.
5. CDC. Classification system for human immunodeficiency virus (HIV) infection in children under 13 years of age. MMWR 1987;36:225-30,235.
6. Hardy AM, Starcher ET, Morgan WM, et al. Review of death certificates to assess completeness of AIDS case reporting. Pub Hlth Rep 1987;102(4):386-91.
7. Starcher ET, Biel JK, Rivera-Castano R, Day JM, Hopkins SG, Miller JW. The impact of presumptively diagnosed opportunistic infections and cancers on national reporting of AIDS [Abstract]. Washington, DC : III International Conference on AIDS, June 1-5, 1987.

APPENDIX I

Laboratory Evidence For or Against HIV Infection

1. **For Infection:**
   When a patient has disease consistent with AIDS:
   a. a serum specimen from a patient ≥15 months of age, or from a child <15 months of age whose mother is not thought to have had HIV infection during the child's perinatal period, that is repeatedly reactive for HIV antibody by a screening test (e.g., enzyme-linked immunosorbent assay [ELISA]), as long as subsequent HIV-antibody tests (e.g., Western blot, immunofluorescence assay), if done, are positive; OR
   b. a serum specimen from a child < 15 months of age, whose mother is thought to have had HIV infection during the child's perinatal period, that is repeatedly reactive for HIV antibody by a screening test (e.g., ELISA), plus increased serum immunoglobulin levels and at least one of the following abnormal immunologic test results: reduced absolute lymphocyte count, depressed CD4 (T-helper) lymphocyte count, or decreased CD4/CD8 (helper/suppressor) ratio, as long as subsequent antibody tests (e.g., Western blot, immunofluorescence assay), if done, are positive; OR
   c. a positive test for HIV serum antigen; OR
   d. a positive HIV culture confirmed by both reverse transcriptase detection and a specific HIV-antigen test or in situ hybridization using a nucleic acid probe; OR
   e. a positive result on any other highly specific test for HIV (e.g., nucleic acid probe of peripheral blood lymphocytes).

2. **Against Infection:**
   A nonreactive screening test for serum antibody to HIV (e.g., ELISA) without a reactive or positive result on any other test for HIV infection (e.g., antibody, antigen, culture), if done.

3. **Inconclusive (Neither For nor Against Infection):**
   a. a repeatedly reactive screening test for serum antibody to HIV (e.g., ELISA) followed by a negative or inconclusive supplemental test (e.g., Western blot, immunofluorescence assay) without a positive HIV culture or serum antigen test, if done; OR
   b. a serum specimen from a child < 15 months of age, whose mother is thought to have had HIV infection during the child's perinatal period, that is repeatedly reactive for HIV antibody by a screening test, even if positive by a supplemental test, without additional evidence for immunodeficiency as described above (in 1.b) and without a positive HIV culture or serum antigen test, if done.

APPENDIX II

Definitive Diagnostic Methods for Diseases Indicative of AIDS

Diseases	Definitive Diagnostic Methods
cryptosporidiosis cytomegalovirus isosporiasis Kaposi's sarcoma lymphoma lymphoid pneumonia or hyperplasia *Pneumocystis carinii* pneumonia progressive multifocal leukoencephalopathy toxoplasmosis	microscopy (histology or cytology).
candidiasis	gross inspection by endoscopy or autopsy or by microscopy (histology or cytology) on a specimen obtained directly from the tissues affected (including scrapings from the mucosal surface), not from a culture.
coccidioidomycosis cryptococcosis herpes simplex virus histoplasmosis	microscopy (histology or cytology), culture, or detection of antigen in a specimen obtained directly from the tissues affected or a fluid from those tissues.
tuberculosis other mycobacteriosis salmonellosis other bacterial infection	culture.

HIV encephalopathy* (dementia)	clinical findings of disabling cognitive and/or motor dysfunction interfering with occupation or activities of daily living, or loss of behavioral developmental milestones affecting a child, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection that could explain the findings. Methods to rule out such concurrent illnesses and conditions must include cerebrospinal fluid examination and either brain imaging (computed tomography or magnetic resonance) or autopsy.
HIV wasting syndrome*	findings of profound involuntary weight loss >10% of baseline body weight plus either chronic diarrhea (at least two loose stools per day for ≥ 30 days) or chronic weakness and documented fever (for ≥ 30 days, intermittent or constant) in the absence of a concurrent illness or condition other than HIV infection that could explain the findings (e.g., cancer, tuberculosis, cryptosporidiosis, or other specific enteritis).

*For HIV encephalopathy and HIV wasting syndrome, the methods of diagnosis described here are not truly definitive, but are sufficiently rigorous for surveillance purposes.

APPENDIX III

Suggested Guidelines for Presumptive Diagnosis of Diseases Indicative of AIDS

Diseases	Presumptive Diagnostic Criteria
candidiasis of esophagus	a. recent onset of retrosternal pain on swallowing; AND b. oral candidiasis diagnosed by the gross appearance of white patches or plaques on an erythematous base or by the microscopic appearance of fungal mycelial filaments in an uncultured specimen scraped from the oral mucosa.
cytomegalovirus retinitis	a characteristic appearance on serial ophthalmoscopic examinations (e.g., discrete patches of retinal whitening with distinct borders, spreading in a centrifugal manner, following blood vessels, progressing over several months, frequently associated with retinal vasculitis, hemorrhage, and necrosis). Resolution of active disease leaves retinal scarring and atrophy with retinal pigment epithelial mottling.
mycobacteriosis	microscopy of a specimen from stool or normally sterile body fluids or tissue from a site other than lungs, skin, or cervical or hilar lymph nodes, showing acid-fast bacilli of a species not identified by culture.
Kaposi's sarcoma	a characteristic gross appearance of an erythematous or violaceous plaque-like lesion on skin or mucous membrane. (Note: Presumptive diagnosis of Kaposi's sarcoma should not be made by clinicians who have seen few cases of it.)
lymphoid interstitial pneumonia	bilateral reticulonodular interstitial pulmonary infiltrates present on chest X ray for ≥2 months with no pathogen identified and no response to antibiotic treatment.
Pneumocystis carinii pneumonia	a. a history of dyspnea on exertion or nonproductive cough of recent onset (within the past 3 months); AND b. chest X-ray evidence of diffuse bilateral interstitial infiltrates or gallium scan evidence of diffuse bilateral pulmonary disease; AND c. arterial blood gas analysis showing an arterial pO_2 of <70 mm Hg or a low respiratory diffusing capacity (<80% of predicted values) or an increase in the alveolar-arterial oxygen tension gradient; AND d. no evidence of a bacterial pneumonia.

toxoplasmosis of the brain	a. recent onset of a focal neurologic abnormality consistent with intracranial disease or a reduced level of consciousness; AND b. brain imaging evidence of a lesion having a mass effect (on computed tomography or nuclear magnetic resonance) or the radiographic appearance of which is enhanced by injection of contrast medium; AND c. serum antibody to toxoplasmosis or successful response to therapy for toxoplasmosis.

APPENDIX 6

CDC CLASSIFICATION SYSTEM
FOR HIV-INFECTED CHILDREN LESS THAN 13 YEARS OF AGE

P-0* Indeterminate infection in perinatally exposed children younger than 15 months of age who have antibody to HIV. (See Table 3)

P-1 Asymptomatic infection

A. Normal immune function
Normal immunoglobulins, complete blood cell count, lymphocyte subsets

B. Abnormal immune function
One or more (unexplained): hypergammaglobulinemia, T-helper lymphopenia, decreased T-helper/T-suppressor ratio, absolute lymphopenia

C. Immune function not tested

P-2 Symptomatic infection (causes other than HIV infection excluded)

A. Nonspecific findings
More than two months' persistence of two or more (unexplained): fever, failure to thrive or >10% weight loss, hepatomegaly, splenomegaly, generalized lymphadenopathy ≥0.5 cm. in two or more sites (bilateral = 1 site), parotitis, diarrhea with three or more loose stools daily persistently or recurrently (two or more episodes with dehydration within 2 months)

B. Progressive neurologic disease
One or more: loss of developmental milestones or intellectual ability; impaired brain growth (acquired microcephaly and/or brain atrophy on scan); progressive symmetric motor deficits with two or more of paresis, abnormal tone, pathologic reflexes, ataxia, or gait disturbance

C. Lymphoid interstitial pneumonitis
Histologically confirmed, with diffuse interstitial and peribronchiolar infiltration of lymphocytes and plasma cells without identifiable pathogen or a chronic pneumonitis with bilateral reticulonodular interstitial infiltrates with or without hilar adenopathy on chest radiography for at least two months, unresponsive to antimicrobial therapy and without other identified pathogen

D. Secondary infectious diseases

D-1++ Those listed in CDC definition of AIDS
Pneumocystis carinii pneumonia (PCP); chronic cyptosporidiosis; disseminated toxoplasmosis (onset after 1 month of age); extraintestinal strongyloidiasis; chronic isosporiasis; candidiasis (esophageal, bronchial, or pulmonary); extrapulmonary cryptococcoses; disseminated histoplasmosis, noncutaneous, extrapulmonary or disseminated mycobacterial infection (species other than leprae); Cytomegalovirus (CMV) infection (onset after 1 month of age); extrapulmonary or disseminated coccidioidomycosis; nocardiosis; progressive multifocal leukoencephalopathy; chronic mucocutaneous or disseminated herpes simplex virus infection (onset after 1 month of age)

D-2 Recurrent serious bacterial infections:
Two or more within two years: sepsis, meningitis, pneumonia, abscess of internal organ, bone or joint infection

D-3 Other infections:
Oral candidiasis (persisting two months), two or more episodes of herpes stomatitis, multidermitomal or disseminated herpes zoster

E. Secondary cancers

E-1 Those listed in CDC definition of AIDS
Kaposi sarcoma, B cell non-Hodgkin lymphoma, primary lymphomas of brain

E-2 Others

F. Other diseases possibly caused by HIV infection
Hepatitis, cardiopathy, nephropathy, hematologic disorder (anemia, thrombocytopenia), dermatologic disease

---

The above is the CDC Classification System extracted from The Journal of Pediatric, January 1989 (Modified from Centers of Disease Control: MMWR 1987;36:225-36)

*Pediatric class: classes are mutually exclusive, P-2 designation if retained even if symptoms resolve
+Subclass: patients may be classified in more than one subclass
++Category

(Rev. 9/25/91)

CDC/NIH Recommended Precautions for Laboratory Work with HIV

Facility	Practices & Procedures	Activities Involving
BL2	BL2	clinical specimens body fluids human/animal tissues infected with HIV
BL2	BL3	growing HIV at research lab-scale growing HIV-producing cell lines working with conc. HIV preparations droplet/aerosol production
BL3	BL3	HIV at industrial-scale levels, large-volume, or high-concentration production and manipulation

Effectively Using Biological Safety Cabinets (BSC)

General Suggestions:

- Keep your laboratory meticulously clean. Minimize storage of boxes and supplies, particularly near the BSC.
- Wash your hands thoroughly before and after working in your BSC. Wearing a clean lab coat and gloves while working in a BSC increases your safety and helps reduce contamination of research materials.
- The effectiveness of the BSC is a function of directional airflow (inward and downward, through a high efficiency filter). Anything that disrupts the airflow pattern reduces cabinet effectiveness, such as: rapidly moving your arms in and out of the BSC; people walking rapidly behind you; down-drafts from ventilation systems; open laboratory doors.
- Understand how the cabinet works. Plan your work. Protect yourself, your research, and your coworkers.

Operational Suggestions:

1. Turn on BSC. Wipe work surface with 70% EtOH. Wipe off each item you need for your procedures and place in cabinet. Allow cabinet to run at least five minutes before beginning work.
   a. Do NOT place objects over the front air intake grille; do NOT block the rear exhaust grille. b. Arrange materials to segregate contaminated and clean items. Minimize movement of contaminated items over clean ones. Remember... "work from clean to dirty."
   b. Work should be performed at least six inches back from the front air intake grille.
2. Put on clean lab coat. Thoroughly wash your hands. Put on gloves, as appropriate.
3. Follow good microbiological techniques, such as holding open tubes and bottles as horizontal as possible.
   a. Use convenient pipetting aids. Do not mouth pipette.
   b. Use horizontal pipette discard pans containing appropriate disinfectant inside BSC. Do not use vertical pipette discard canisters on floor outside cabinet.

c. It is not necessary to flame items. The flame creates turbulence in airflow and will compromise sterility; heat buildup may damage the filters.

4. If you need to remove items from the BSC or introduce new items, move your arms slowly in and out of the cabinet to minimize disruption of the airflow.

5. If you use a piece of equipment that creates air turbulence in the BSC (such as a centrifuge, blender, sonicator), place the equipment in the back 1/3 of the cabinet; stop other work while equipment is operating.

6. Protect the building vacuum system from biohazards by placing a cartridge filter between the vacuum trap and the source valve in the cabinet.

7. Clean up all spills in the cabinet immediately. Wait 3-5 minutes before resuming work if procedures allow.

8. Remove all materials and wipe all interior surfaces with 70% alcohol when you are finished work. Let cabinet run 10 minutes, turn off. Examine the tray under the work surface, disinfecting and cleaning as necessary.

9. Discard waste materials appropriately (autoclave, MPW, etc.).

10. Remove lab coat and wash hands thoroughly before leaving laboratory

CENTERS FOR DISEASE CONTROL

August 21, 1987 / Vol. 36 / No. 2S

MMWR

Supplement

MORBIDITY AND MORTALITY WEEKLY REPORT

Survival of HIV in the Environment

The most extensive study on the survival of HIV after drying involved greatly concentrated HIV samples, i.e., 10 million tissue-culture infectious doses per milliliter (*31*). This concentration is at least 100,000 times greater than that typically found in the blood or serum of patients with HIV infection. HIV was detectable by tissue-culture techniques 1-3 days after drying, but the rate of inactivation was rapid. Studies performed at CDC have also shown that drying HIV causes a rapid (within several hours) 1-2 log (90%-99%) reduction in HIV concentration. In tissue-culture fluid, cell-free HIV could be detected up to 15 days at room temperature, up to 11 days at 37 C (98.6 F), and up to 1 day if the HIV was cell-associated.

When considered in the context of environmental conditions in health-care facilities, these results do not require any changes in currently recommended sterilization, disinfection, or housekeeping strategies. When medical devices are contaminated with blood or other body fluids, existing recommendations include the cleaning of these instruments, followed by disinfection or sterilization, depending on the type of medical device. These protocols assume "worst-case" conditions of extreme virologic and microbiologic contamination, and whether viruses have been inactivated after drying plays no role in formulating these strategies. Consequently, no changes in published procedures for cleaning, disinfecting, or sterilizing need to be made.

Cleaning and Decontaminating Spills of Blood or Other Body Fluids

Chemical germicides that are approved for use as "hospital disinfectants" and are tuberculocidal when used at recommended dilutions can be used to decontaminate spills of blood and other body fluids. Strategies for decontaminating spills of blood and other body fluids in a patient-care setting are different than for spills of cultures or other materials in clinical, public health, or research laboratories. In patient-care areas, visible material should first be removed and then the area should be decontaminated. With large spills of cultured or concentrated infectious agents in the laboratory, the contaminated area should be flooded with a liquid germicide before cleaning, then decontaminated with fresh germicidal chemical. In both settings, gloves should be worn during the cleaning and decontaminating procedures.

NOTE: Only those sections of critical relevance to laboratory workers are included in this document. Refer to the August 21, 1987 Supplement of MMWR if interested in reviewing the entire supplementary issue.

Management of Exposures

If a health-care worker has a parenteral (e.g., needlestick or cut) or mucous-membrane (e.g., splash to the eye or mouth) exposure to blood or other body fluids or has a cutaneous exposure involving large amounts of blood or prolonged contact with blood—especially when the exposed skin is chapped, abraded, or afflicted with dermatitis—the source patient should be informed of the incident and tested for serologic evidence of HIV infection after consent is obtained. Policies should be developed for testing source patients in situations in which consent cannot be obtained (e.g., an unconscious patient).

If the source patient has AIDS, is positive for HIV antibody, or refuses the test, the health-care worker should be counseled regarding the risk of infection and evaluated clinically and serologically for evidence of HIV infection as soon as possible after the exposure. The health-care worker should be advised to report and seek medical evaluation for any acute febrile illness that occurs within 12 weeks after the exposure. Such an illness—particularly one characterized by fever, rash, or lymphadenopathy—may be indicative of recent HIV infection. Seronegative health-care workers should be retested 6 weeks post-exposure and on a periodic basis thereafter (e.g., 12 weeks and 6 months after exposure) to determine whether transmission has occurred. During this follow-up period—especially the first 6-12 weeks after exposure, when most infected persons are expected to seroconvert—exposed health-care workers should follow U.S. Public Health Service (PHS) recommendations for preventing transmission of HIV (*36,37*).

No further follow-up of a health-care worker exposed to infection as described above is necessary if the source patient is seronegative unless the source patient is at high risk of HIV infection. In the latter case, a subsequent specimen (e.g., 12 weeks following exposure) may be obtained from the health-care worker for antibody testing. If the source patient cannot be identified, decisions regarding appropriate follow-up should be individualized. Serologic testing should be available to all health-care workers who are concerned that they may have been infected with HIV.

If a patient has a parenteral or mucous-membrane exposure to blood or other body fluid of a health-care worker, the patient should be informed of the incident, and the same procedure outlined above for management of exposures should be followed for both the source health-care worker and the exposed patient.

References

31. Resnik L, Veren K, Salahuddin SZ, Tondreau S, Markham PD. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. JAMA 1986;255:1887-91.

36. CDC. Prevention of acquired immune deficiency syndrome (AIDS): Report of inter-agency recommendations. MMWR 1983;32:101-3.

37. CDC. Provisional Public Health Service inter-agency recommendations for screening donated blood and plasma for antibody to the virus causing acquired immunodeficiency syndrome. MMWR 1985;34:1-5.

APPENDIX 7

CONSENT FORM
MATERNAL CONSENT - PRENATAL STUDIES

NAME OF THIS STUDY:

Pulmonary and Cardiovascular Complications of Vertically Transmitted Human Immunodeficiency Virus (HIV) Infection.

PURPOSE OF THIS STUDY:

HIV infects and damages certain blood cells which are part of the body's immune system. The damage to the body's immune system can result in the development of common and unusual infections and/or unusual forms of cancer. The virus can also infect the heart and lungs causing damage. While therapies exist for many, but not all of the infections which might occur, there is no known cure for the immunologic defects caused by HIV. Infection with this virus has been associated with the development of the acquired immunodeficiency syndrome (AIDS) in some patients, while infection in other patients has not led to any illness over the period of time they have been observed. It is impossible at this time to predict which patients infected with HIV will go on to develop serious illness.

You have been referred to us because you are infected with the human immunodeficiency virus and are pregnant. Your child may become infected with HIV while developing in your uterus. Although it is not clear as to the percent of children who may be infected during pregnancy, some studies suggest that it may be as high as 30%.

Because you are infected with HIV and are pregnant, you are being offered the opportunity to allow your child to participate in this study to determine how the virus affects the heart and lungs from the fetal period through the first four to five years of life. Your child will still continue to receive medical treatment as determined by the AIDS specialists at _____ hospital. It is hoped that this study will enable us to determine at the earliest possible time, heart and lung involvement from the HIV virus and allow for early treatment and improved health.

SPONSORSHIP:

Drs._____ and _____ are conducting this study under the general supervision of the _____ Hospital and in collaboration with _____. These institutions along with five other major medical centers across the country are under the sponsorship of the National Heart, Lung and Blood Institute of the National Institute of Health.

PROCEDURES:

If you agree to enroll your fetus/child in this study, the following will happen:

1) Ultrasound examination of your fetus' heart and lungs will be performed two times during your pregnancy.

2) Your child will be examined during the first week of life and every three to four months thereafter.

3) Every four months your child will have a chest x-ray, electrocardiogram (to examine the electroactivity of the heart), hemoglobin oxygen saturation (tapes applied to the skin to measure the amount of oxygen in the blood stream), and an echocardiogram (sound wave test of the heart). These studies will take about two to three hours.

4) Every six months your child will come to the Hospital for a series of tests lasting about four to five hours. These will include:

   a) Pulmonary function testing: (Your child will be given mild sedation, and measurements of the air he/she breaths will be taken using a small mask over your child's nose and mouth.) This measures how well the lung functions.

   b) Technetium aerosol scan (DTPA): (Your child will breath through a mask containing a safe, small amount of radioactively labelled material.) Your child's chest will then be scanned with a machine to detect whether there is injury to the lung.

5) The investigators will check your child's records to gather information about other regular blood tests.

Participation in this study will take about one day every three to four months.

RISKS AND DISCOMFORTS:

1) Arterial puncture: The risk of drawing arterial blood includes temporary discomfort from the needle stick, bruising, bleeding, or infection.

2) Radiation: Although radiation may be potentially harmful, the amount child will be exposed to is relatively small and the risks of this exposure are too small to measure. If your child has already had many x-rays, you should discuss this with the investigator before agreeing to have your child participate in this study.

3) Sedation: In order to have pulmonary function tests and echocardiograms, your child will need to be sedated. Chloral Hydrate will be given by mouth or suppository. This will cause drowsiness for several hours. If she/he experiences any unusual reaction, a different type of sedation will be used for the future studies.

4) There is no known risk to the fetus with fetal ultrasound.

BENEFITS:

It is hoped that information gained from this study will show when the AIDS virus affects the heart and lungs, as early as possible, so that treatment can begin to avoid sickness and/or progression. Information gained from this study will help doctors learn more about HIV infection in infants and children and how to treat it.

ALTERNATIVES:

If you choose not to have your child participate in this study, your child will still be seen by the doctors at _____.

CONFIDENTIALITY:

_____ Hospital will protect the confidentiality of the medical records of you and your child. In order to verify the study data, officials from the National Institute of Health, National Heart, Lung and Blood Institute, may need to see some specified records including those of your child. However, neither NHLBI nor the Clinical Coordinating Center will receive any information which would allow the identification of either you or your child.

TREATMENT AND COMPENSATION FOR INJURY:

If your child is injured as a result of being in this study, medical care will be available at _____ Hospital. However, _____ Hospital does not provide compensation or free hospital services for such injury. Further information concerning the treatments available or the matter of compensation may be obtained from Dr. _____ or Dr. _____.

REIMBURSEMENTS:

In return for your expenses incurred travelling here for visits, you will be reimbursed a small amount to cover travel expenses.

QUESTIONS:

Before you agree to participate you should talk with Dr._____ or Dr. _____, who will answer your questions. If you have any other questions during the course of this study, you may call Dr. _____ _____ at _____, or Dr. _____ at _____.

In addition, if you wish to speak to an impartial third party who is not involved with the project and who may be contacted to discuss concerns or complaints, you may contact:

_____

_____

_____

CONSENT TO BE A RESEARCH PARTICIPANT AND LIST OF RIGHTS:

You will be given a copy of this form and a copy of the "List of Rights of a Participant in a Medical Experiment" to keep.

PARTICIPATION IN RESEARCH IS VOLUNTARY:

If any significant new findings are developed during the course of the research which may affect your willingness to have your child continue involvement, we will notify you as quickly as possible. If you are having difficulty complying with the every three month visits so that the investigators cannot collect accurate data, your child's participation in the study may be terminated by the investigators. You have the right to refuse to have your child take part in this study. You may withdraw your child at any time without jeopardizing your child's medical care at _____ Hospital. If you wish to allow your child to participate, please sign this form.

_____ _____

Date Parent's or Guardian's Signature

_____ _____

Date Parent's or Guardian's Signature

_____ _____

Date Witness

CONSENT FORM
GROUP II PATIENTS POSTNATAL ENROLLMENT

NAME OF THIS STUDY:

Pediatric Pulmonary and Cardiovascular Complications of Vertically Transmitted Human Immunodeficiency Virus (HIV) Infection.

PURPOSE OF THIS STUDY:

HIV infects and damages certain blood cells which are part of the body's immune system. The damage to the body's immune system can result in the development of common and unusual infections and/or unusual forms of cancer. The virus can also infect the heart and lungs causing damage. While therapies exist for many, but not all of the infections which might occur, there is no known cure for the immunologic defects caused by HIV. Infection with this virus has been associated with the development of the acquired immunodeficiency syndrome (AIDS) in some patients, while infection in other patients has not led to any illness over the period of time they have been observed. It is impossible at this time to predict which patients infected with HIV will go on to develop serious illness.

Your child was referred to us because he/she is less than 14 days of age and you are infected with the human immunodeficiency virus. A child may become infected with HIV while developing in the uterus. Although it is not clear as to the percent of children who may be infected during pregnancy, some studies suggest that it may be as high as 30%. Because of this, your child is eligible to participate in this study to determine how the virus affects the heart and lungs from the newborn period through the first four to five years of life. Your child will still continue to receive medical treatment as determined by the AIDS specialists at _____ hospital. It is hoped that this study will enable us to determine at the earliest possible time, heart and lung involvement from the HIV virus and allow for early treatment and improved health.

SPONSORSHIP:

Drs._____ and _____ are conducting this study under the general supervision of the _____ Hospital and in collaboration with _____. These institutions along with five other major medical centers across the country are under the sponsorship of the National Heart, Lung and Blood Institute of the National Institute of Health.

PROCEDURES:

If you agree to enroll your child in this study, the following will happen:

1) Your child will be examined at the time of enrollment and every three to four months thereafter.

2) Every four months your child will have a chest x-ray, electrocardiogram (to examine the electroactivity of the heart), hemoglobin oxygen saturation (tapes applied to the skin to measure the amount of oxygen in the blood stream), and an echocardiogram (sound wave test of the heart.) These studies will take about two to three hours.

3) Every six months your child will come to the Hospital for a series of tests lasting about four to five hours. These will include:

   a) Pulmonary function testing: (Your child will be given mild sedation, and measurements of the air he/she breathes will be taken using a small mask over your child's nose and mouth.) This measures how well the lung functions.

   b) Technetium aerosol scan (DTPA): (Your child will breath through a mask containing a safe, small amount of radioactively labelled material.) Your child's chest will then be scanned with a machine to detect whether there is injury to the lung.

4) The investigators will check your child's records to gather information about other regular blood tests.

Participation in this study will take about one day every three to four months.

RISKS AND DISCOMFORTS:

1) Arterial puncture: The risk of drawing arterial blood includes temporary discomfort from the needle stick, bruising, bleeding, or infection.

2) Radiation: Although radiation may be potentially harmful, the amount child will be exposed to is relatively small and the risks of this exposure are too small to measure. If your child has already had many x-rays, you should discuss this with the investigator before agreeing to have your child participate in this study.

3) Sedation: In order to have pulmonary function tests and echocardiograms, your child will need to be sedated. Chloral Hydrate will be given by mouth or suppository. This will cause drowsiness for several hours. If she/he experiences any unusual reaction, a different type of sedation will be used for the future studies.

BENEFITS:

It is hoped that information gained from this study will show when the AIDS virus affects the heart and lungs, as early as possible, so that treatment can begin to avoid sickness and/or progression. Information gained from this study will help doctors learn more about HIV infection in infants and children and how to treat it.

ALTERNATIVES:

If you choose not to have your child participate in this study, your child will still be seen by the doctors at _____.

CONFIDENTIALITY:

_____ Hospital will protect the confidentiality of the medical records of you and your child. In order to verify the study data, officials from the National Institute of Health, National Heart, Lung and Blood Institute, may need to see some specified records including those of your child. However, neither NHLBI nor the Clinical Coordinating Center will receive any information which would allow the identification of either you or your child.

TREATMENT AND COMPENSATION FOR INJURY:

If your child is injured as a result of being in this study, medical care will be available at _____ Hospital. However, _____ Hospital does not provide compensation or free hospital services for such injury. Further information concerning the treatments available or the matter of compensation may be obtained from Dr. _____ or Dr. _____.

REIMBURSEMENTS:

In return for your expenses incurred travelling here for visits, you will be reimbursed a small amount to cover travel expenses.

QUESTIONS:

Before you agree to participate you should talk with Dr._____ or Dr. _____, who will answer your questions. If you have any other questions during the course of this study, you may call Dr. _____ _____ at _____, or Dr. _____ at _____.

In addition, if you wish to speak to an impartial third party who is not involved with the project and who may be contacted to discuss concerns or complaints, you may contact:

_____

_____

_____

CONSENT TO BE A RESEARCH PARTICIPANT AND LIST OF RIGHTS:

You will be given a copy of this form and a copy of the "List of Rights of a Participant in a Medical Experiment" to keep.

PARTICIPATION IN RESEARCH IS VOLUNTARY:

If any significant new findings are developed during the course of the research which may affect your willingness to have your child continue involvement, we will notify you as quickly as possible. If you are having difficulty complying with the every three month visits so that the investigators cannot collect accurate data, your child's participation in the study may be terminated by the investigators. You have the right to refuse to have your child take part in this study. You may withdraw your child at any time without jeopardizing your child's medical care at _____ Hospital. If you wish to allow your child to participate, please sign this form.

_____ _____

Date Parent's or Guardian's Signature

_____ _____

Date Parent's or Guardian's Signature

_____ _____

Date Witness

APPENDIX IV

Equivalent Terms and International Classification of Disease (ICD) Codes for AIDS-Indicative Lymphomas

The following terms and codes describe lymphomas indicative of AIDS in patients with antibody evidence for HIV infection (Section II.A.8 of the AIDS case definition). Many of these terms are obsolete or equivalent to one another.

ICD-9-CM (1978)

Codes	Terms
200.0	Reticulosarcoma lymphoma (malignant): histiocytic (diffuse) reticulum cell sarcoma: pleomorphic cell type or not otherwise specified
200.2	Burkitt's tumor or lymphoma malignant lymphoma, Burkitt's type

ICD-O (Oncologic Histologic Types 1976)

Codes	Terms
9600/3	Malignant lymphoma, undifferentiated cell type non-Burkitt's or not otherwise specified
9601/3	Malignant lymphoma, stem cell type stem cell lymphoma
9612/3	Malignant lymphoma, immunoblastic type immunoblastic sarcoma, immunoblastic lymphoma, or immunoblastic lymphosarcoma
9632/3	Malignant lymphoma, centroblastic type diffuse or not otherwise specified, or germinoblastic sarcoma: diffuse or not otherwise specified
9633/3	Malignant lymphoma, follicular center cell, non-cleaved diffuse or not otherwise specified
9640/3	Reticulosarcoma, not otherwise specified malignant lymphoma, histiocytic: diffuse or not otherwise specified reticulum cell sarcoma, not otherwise specified malignant lymphoma, reticulum cell type
9641/3	Reticulosarcoma, pleomorphic cell type malignant lymphoma, histiocytic, pleomorphic cell type reticulum cell sarcoma, pleomorphic cell type
9750/3	Burkitt's lymphoma or Burkitt's tumor malignant lymphoma, undifferentiated, Burkitt's type malignant lymphoma, lymphoblastic, Burkitt's type

Morbidity and Mortality Weekly Report is prepared by the Centers for Disease Control, Atlanta, Georgia. This edition is printed and distributed by the Massachusetts Medical Society, publishers of *The New England Journal of Medicine*. The data in this report are provisional, based on weekly reports to CDC by state health departments. The reporting week concludes at close of business on Friday; compiled data on a national basis are officially released to the public on the succeeding Friday.

The editor welcomes accounts of interesting cases, outbreaks, environmental hazards, or other public health problems of current interest to health officials. Such reports and any other matters pertaining to editorial or other textual considerations should be addressed to the attention of the Editor, *Morbidity and Mortality Weekly Report*, Centers for Disease Control, Atlanta, Georgia 30333.

U.S. Department of Health and Human Services / Public Health Service
Director, Centers for Disease Control: James O. Mason, M.D., Dr.P.H.
Director, Epidemiology Program Office: Carl W. Tyler, Jr., M.D.
Editor: Michael B. Gregg, M.D.
Managing Editor: Gwendolyn A. Ingraham

HHS Publication No. (CDC) 85-8017

Subscriptions to this edition of *Morbidity and Mortality Weekly Report* are available through the Massachusetts Medical Society, C.S.P.O. Box 9120, Waltham, MA 02254-9120, U.S.A. *MMWR* also expects to publish four Quarterly Surveillance Reports and two to four Supplements during the course of a yearly subscription.

SUBSCRIPTION RATES

Third Class Mail: 52 issues and annual and supplements, $26.00
First Class Mail: 52 issues and annual and supplements, $46.00

Please inquire about our foreign and Canadian rates.

Reprints of articles in *MMWR* may be ordered in minimum quantities of 100. Also available are single copies and back issues of *MMWR*, the Quarterly Surveillance Reports, the Supplements, and the Annual Reports at the following domestic rates:

Weekly issue	$ 2.00
Quarterly Surveillance Reports	3.00
Supplements	3.00
Annual Report	4.00

Please inquire for more information and for foreign rates.

Massachusetts Medical Society
C.S.P.O. Box 9120
Waltham, MA 02254-9120
Address correction requested

APPENDIX 8

(Rev. 6/1/92)

GROUP I: ROUTINE SCREENING

	TIMING (MONTHS FROM ON-STUDY)																			
TEST	0	3	4	6	8	9	12	16	18	20	24	28	30	32	36	40	42	44	48	52
ELISA																				
W. BLOT							X				X				X				X	
HIV CULTURE																				
SERUM IG	X						X				X				X				X	
CD 3/4/8	X						X				X				X				X	
DHST(1)	X						X				X				X				X	
CMV CULTURE(2)	X						X				X				X				X	
CMV SEROLOGY(2)	X						X				X				X				X	
EBV CULTURE(2)	X						X				X				X				X	
EBV SEROLOGY(2)	X						X				X				X				X	
CBC	X						X				X				X				X	
ESR	X						X				X				X				X	
LDH	X						X				X				X				X	
SERUM STORAGE	X						X				X				X				X	
SPO2	X	X		X		X	X		X		X		X		X		X		X	
CXR	X						X				X				X				X	
PFT	X			X			X		X		X		X		X		X		X	
DTPA	X			X			X		X		X									
EKG	X						X				X				X				X	
HOLTER	X						X				X				X				X	
ECHO	X		X		X		X	X		X	X	X		X	X	X		X	X	X

FOOTNOTES:
(1) Children 1 year and older
(2) These tests are done only when clinically indicated.

GROUP IIa: ROUTINE SCREENING

(Rev. 6/1/92)

	TIMING (MONTHS FROM ON-STUDY)																					
TEST	0	3	4	6	8	9	12	15	16	18	20	21	24	28	30	32	36	40	42	44	48	52
ELISA	X						X			X												
W. BLOT	X						X			X			X				X				X	
HIV CULTURE				X			X															
SERUM IG	X			X			X			X			X		X		X		X		X	
CD 3/4/8	X	X				X		X				X			X		X		X		X	
DHST							X						X				X				X	
CMV CULTURE(*)	X			X			X			X			X		X		X		X		X	
CMV SEROLOGY(*)				X			X			X			X		X		X		X		X	
EBV CULTURE	X			X			X			X			X		X		X		X		X	
EBV SEROLOGY				X			X			X			X		X		X		X		X	
CBC	X			X			X			X			X		X		X		X		X	
ESR				X			X			X			X		X		X		X		X	
LDH	X			X			X			X			X		X		X		X		X	
SERUM STORAGE	X			X			X			X			X		X		X		X		X	
SPO2	X	X		X		X	X			X			X		X		X		X		X	
CXR		X				X				X					X				X			
PFT				X			X			X			X		X		X		X		X	
DTPA				X			X			X			X		X		X					
EKG	X						X						X				X				X	
HOLTER	X						X						X				X				X	
ECHO	X		X		X		X		X		X		X	X		X	X	X		X	X	X
FETAL ECHO	X																					

* CMV Testing discontinued when child is positive for CMV infection.

GROUP IIb: ROUTINE SCREENING

	TIMING FROM BIRTH (MOS.)									
TEST	24	28	30	32	36	40	42	44	48	52
ELISA	X				X				X	
W. BLOT										
HIV CULTURE										
SERUM IG	X		X		X		X		X	
CD 3/4/8			X		X		X		X	
DHST	X				X				X	
CMV CULTURE(*)	X		X		X		X		X	
CMV SEROLOGY(*)	X		X		X		X		X	
EBV CULTURE	X		X		X		X		X	
EBV SEROLOGY	X		X		X		X		X	
CBC	X		X		X		X		X	
ESR	X		X		X		X		X	
LDH	X		X		X		X		X	
SERUM STORAGE	X		X		X		X		X	
SPO2	X		X		X		X		X	
CXR										
PFT	X		X		X		X		X	
DTPA										
EKG	X				X				X	
HOLTER	X				X				X	
ECHO	X	X		X	X	X		X	X	X
FETAL ECHO										

* CMV Testing discontinued when child is positive for CMV infection.

(Rev. 6/1/92)

(Rev. 6/1/92)

GROUP II MOTHERS: ROUTINE SCREENING

TIMING FROM BIRTH	0
ELISA	
W. BLOT	
HIV CULTURE	
SERUM IG	
CD3/4/8	x
DHST	
CMV CULTURE	x
CMV SEROLOGY	x
EBV CULTURE	x
EBV SEROLOGY	x
CBC	
ESR	
LDH	
SERUM STORAGE	
SPO2	
CXR	
PFT	
DTPA	
EKG	
HOLTER	
ECHO	
FETAL ECHO	