MOTHER TO CHILD TRANSMISSION OF HIV AND ITS PREVENTION WITH AZT AND NEVIRAPINE

A CRITICAL ANALYSIS OF THE EVIDENCE

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PROLOGUE

"We absolutely must leave room for doubt or there is no progress and there is no learning. There is no learning without having to pose a question".

Richard P Feynman, Physicist and Nobel Laureate. Galileo Symposium Address 1964.

The impetus for this review arose as a result of the Presidential AIDS Panel meetings held in South Africa during May and July 2000 under the auspices of the South African Government and President Thabo Mbeki. Our purpose in this publication was not to discuss the HIV theory of AIDS or even the existence of HIV (Those interested in the fundamental question of proving the existence of HIV will find it discussed in Appendix XI). The question this monograph addresses, assuming that HIV does exist, is whether a unique retrovirus is transmitted from pregnant women to their children and whether compounds such as zidovudine (AZT) and nevirapine are able to prevent such transmission.

In Parts I-II we examine the indirect methods said to prove HIV infection and transmission, as well as epidemiological data on mother-to-child transmission. Part III analyses data associated with breastfeeding practices and the possibility of infection. Part IV includes pharmacological data relevant to zidovudine and nevirapine as well as their effects on the several parameters claimed to be indicative of HIV infection and transmission. Included in Part IV is a detailed review of the Pediatric AIDS Clinical Trials Group (ACTG) 076 study which forms the basis of recommending the administration of AZT to all pregnant, HIV positive women and their newborn babies. In Part V we present data on non-retroviral factors which affect the putative mother-to-child transmission of HIV and its prevention, especially the role of nutrition including micronutrients. Part VI consists of a general discussion of the topic.

In reviewing evidence of such a voluminous nature authors face the perennial problem of space and balance. To present too much data is to overwhelm the reader. To present too little is to risk scientific scholarship. Notwithstanding, given the critical nature of this subject to continents of people, and that mother to child transmission is accepted as fact by virtually the whole scientific establishment, we decided to present and discuss at length all the data we could muster. However, with the reader in mind, many of the epidemiological studies are prefaced with a *precis*. We make apology for studies we may have inadvertently omitted.

Scientists who question prevailing theories are under an obligation to present alternatives or, as a minimum, explain particular observations by other means. Consequently, we have included data on the role of cellular oxidation in the genesis of “HIV” phenomenology as well as diseases constituting the clinical syndrome.

It is hoped that this critical analysis of the evidence will prompt a reappraisal of the data interpreted as proof of mother to child transmission of HIV and thereby direct resources towards appropriate efforts to ameliorate factors linked to such biological phenomena.

PART I
TESTS USED TO DETERMINE HIV INFECTION

1.1 Introduction

In 1994 a paper was published by researchers from the UK, Tanzania and the United Nations Children Fund stating "It has been estimated that by the end of 1992, one million HIV-1 infected children had been born in Africa, 600,000 of whom have progressed to AIDS".[1] In 1999 researchers from France and Rwanda wrote that "According to the World Health Organisation (WHO), 1.1 million children were living with human immunodeficiency virus (HIV) infection worldwide at the end of 1997. Of these, the great majority live in sub-Saharan Africa and were infected by their mothers during pregnancy, delivery or breastfeeding".[2] In the same year, two researchers from the University of Zimbabwe wrote: "It is a well established fact that infants born to HIV positive mothers are at risk of acquiring HIV infection from their mothers. HIV infected newborns and infants have a poorer prognosis than those not infected, and therefore, the already existing high infant mortality may significantly rise in sub-Saharan Africa when considering the maternal-foetal transmission rate of 25-35%. With the use of anti-retroviral therapy, HIV transmission to an unborn foetus during pregnancy may be significantly reduced".[3] In 2000 Helene Gayle from the CDC wrote "According to estimates of the global HIV/AIDS situation as of the end of 1999 from the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO), more than 47 million adults and children have been infected with HIV since the beginning of the pandemic, approximately 34.3 million are living with HIV infection or AIDS, and nearly 15,000 people, both adults and children, become infected each day. In 1999, an estimated 5 million people became infected including 620,000 children aged less than 15 years".[4] For such claims to be made by so many leading researchers evidence must exist which proves mother-to-child transmission (MCT) of HIV as well as its inhibition by compounds such as 3'-azido-3'-deoxythymidine (zidovudine, AZT) and nevirapine.

To prove that mother-to-child transmission of HIV takes place first one must have proof that HIV exists. Assuming that HIV does exist then, to prove MCT one must have specific tests to determine the infectious status of both mother and child. At present, infection of the mother is determined by antibody tests and that of the child by antibody tests, "HIV isolation" and measurements of “HIV” RNA or DNA utilising the polymerase chain reaction (PCR). In addition, in many studies children are declared infected with HIV by transmission from their mothers without any laboratory tests but solely if they satisfy clinical criteria encompassed by the Bangui AIDS definition for children in Africa,[5] the Ghent definitions,[6] the CDC 1987[7] or the 1994 CDC revised definition for children aged less than thirteen years[8] (Appendices I-IV).

1.2 Antibody Tests

Antibodies are proteins synthesised in the body as a result of exposure to foreign substances, including infectious agents and proteins, collectively known as antigens (ANTIbody GENerating). Antibodies react with antigens and, assuming the reaction is specific, this property may be used to detect either reactant if the identity of the other is known. Thus, to perform an HIV antibody test to prove a viral infection, two things are required. A sample of blood from the individual thought infected and a test kit containing the virus or its proteins. To date nobody has performed HIV antibody tests using the HIV particles. Rather all the antibody test kits are prepared using approximately ten proteins which the HIV experts claim are those of a unique retrovirus, "HIV". There are two "HIV" antibody tests in common use, the ELISA and Western Blot (WB). The ELISA causes a colour change when a mixture of the "HIV" proteins reacts with antibodies in serum from a patient. In the Western Blot the "HIV" proteins are first separated along the length of a nitrocellulose strip. This enables individual reactions between antibodies and the "HIV" proteins to be visualised as a series of darkened "bands". These bands are referred to with a small ‘p’ (for protein) followed by a number designating the protein’s molecular weight in thousands, for example, p24. In the majority of countries/institutions/laboratories the diagnosis of HIV infection consists of performing an initial ELISA which, if reactive, is repeated. If the ELISA is repeatedly reactive a Western blot is then performed because virtually all experts agree that the ELISA is insufficiently specific. In other words and according to the same experts, the Western blot distinguishes “true” from “false” reactivity in repeatedly reactive ELISAs because, unlike the ELISA, the Western blot assay is highly specific.

It is manifestly obvious that the only way to claim a protein is a viral component is to extract it from a viral particle. However, a single virus particle is a microscope entity of the order of 1/10,000 of millimetre in size and thus it is impossible to obtain proteins in such a manner. The next best thing is to obtain the proteins from material consisting of

PART II
EPIDEMIOLOGICAL EVIDENCE FOR MOTHER TO CHILD TRANSMISSION OF HIV

2.1 Introduction

The way to obtain the most reliable epidemiological evidence of mother to child transmission is to conduct prospective, randomised, blinded, controlled studies. The women who act as controls should, with the one exception of HIV seropositivity, be identical with the test women. In other words, a scientific study should consist of two groups of mother-child pairs incorporating:

(a) children whose mothers are HIV positive;

(b) children whose mothers are HIV negative but are otherwise identical with the HIV positive women.

All the tests and clinical observations must be performed blindly in children born to both groups of mothers.

2.2 Studies from the USA

Children of drug using or economically disadvantaged mothers are of low birthweight and develop immune deficiency and a range of diseases.

In 1982 the CDC reported 4 cases of "Unexplained immunodeficiency and opportunistic infections in infants—New York, New Jersey, California".[201] At three months of age a Black/Hispanic infant of an intravenous (IV) drug user mother developed oral candidiasis. This was followed by hepatosplenomegaly and staphylococcal impetigo. "Growth, which had been slow, stopped at 9 months". At 17 months of age the infant had progressive pulmonary infiltrates and oral candidiasis and "Mycobacterium avium-intracellulare was cultured from sputum and bone samples". T-cell studies that were initially normal, at 20 months "showed lymphopenia, decreased number of T-lymphocytes, and severely impaired T-cell function in vitro".

A Haitian infant with retarded physical development, developed diarrhoea at 2 weeks and at 5 months was hospitalised because of fever and diarrhoea. He had hepatosplenomegaly, lymphadenopathy and otitis media. While on treatment, he developed pulmonary infiltrates. "An open lung biopsy revealed Pneumocystis carinii, Cryptococcus neoformans, and cytomegalovirus. Serum IgG, IgA and IgM concentration were elevated. The percentage of T-lymphocytes was decreased, but T-cell response to mitogen was normal...The infant died of respiratory insufficiency at 7 months of age...His parents were residents of Brooklyn, New York; their health status is unknown".

A Haitian infant was hospitalised at 5 months with fever and respiratory distress. "Despite antibiotic therapy, the infant's condition deteriorated and an open lung biopsy revealed PCP. Immunological studies showed elevated serum concentration of IgG, IgA and IgM, decreased percentage of T-lymphocytes and impaired T-cell function in vitro". The infant died. The parents' "health status is unknown".

The infant of a white prostitute and IV drug user with a history of oral candidiasis developed vaginal and oral candidiasis at 2 months of age, which responded to therapy. At "5 months, candidiasis recurred and she had hepatosplenomegaly. Immunological evaluation showed that serum IgG, IgA, and IgM levels, normal at 2 months, were now elevated. The percentage of T-lymphocytes was decreased and lymphocyte response to alloantigens was impaired. At 6 months of age, the infant was hospitalised because of fever and cough. Open lung biopsy revealed PCP. Despite appropriate antibiotic therapy, she died".

The above 4 cases were summarised as follows: "It is possible that these infants had the acquired immune deficiency syndrome (AIDS)…Although the aetiology of AIDS remains unknown, a series of epidemiologic observations suggests it is caused by an infectious agent. If the infants described in the four case reports had AIDS, exposure to the putative "AIDS agent" must have occurred very early. Cases 2-4 were less than 6 months old when they had serious opportunistic infections. Case 1 had oral candidiasis beginning at 3 months of age, although M. avium-intracellulare infection was not documented until 17 months. Transmission of an "AIDS agent" from mother to child, either in utero or shortly after birth, could account for the early onset of immunodeficiency in these infants".[201]

Immune deficiency and illness in Black and Hispanic children. Parenteral drug use, neglect and malnutrition or an infectious agent?

In 1983 researchers from New Jersey published a paper entitled "Immune Deficiency Syndrome in Children" They described clinical and immunological findings in 8 infants/children (5 black, 2 black/hispanic, 1 hispanic). The mothers of 4 of them and the fathers of another 2 admitted to IV drug use. All the children had interstitial pneumonia, 4 had

PART III
BREAST FEEDING AND TRANSMISSION OF HIV

3.1 Introduction

There is unquestionable evidence that breast-feeding protects babies against morbidity and mortality from infectious diseases.[291] "Breast-feeding provides protection to babies in many ways. It provides ideal nutrition to the infant at no cost and gives an immunologic protection against agents responsible for diarrheal and respiratory diseases, as well as other infections. Breast-feeding also plays an important role in birth spacing, mainly in the developing world. Finally, breast-feeding is important as favoring mother-child interactions and the psychosocial development of the child".[292] Given the importance of breast-feeding for the child's well being one would need to have extremely well founded reasons to advise against it. The minimum absolutely necessary but not sufficient reasons for advising mothers against breast-feeding their infants because of HIV is proof that:

1. HIV exists.

2. HIV is present in the milk of infected women.

3. HIV can be transmitted to the child by breast-feeding.

To prove this one must conduct prospective, randomised, blinded, controlled studies in which the possibility of HIV infection by means other than breastfeeding has been rigorously excluded.

3.2 HIV in Breast Milk

Assuming the existence of HIV is proven the only way to prove the presence of HIV in breast milk is to isolate it from breast milk. In this regard, from 1985 till the present, HIV scientists cite two publications. The first was published in 1985 by researchers from Belgium, including Institut Pasteur du Brabant where the authors claim isolation of HIV from the milk of three healthy women. The first woman was born in Zaire and had a positive ELISA. The second woman was born in Belgium but she and her husband had lived in Zaire during the previous ten years. Her ELISA was negative. The third woman was from Rwanda and she had a positive ELISA. The lymphocytes from these women as well as the child of the first and third were stimulated with PHA and cultured for four weeks with interleukin-2 (IL-2). They were then reacted with "rabbit hyperimmune serum against HTLV-III" (HIV). The authors also mention that the supernatants were tested for reverse transcriptase but only the reaction with the rabbit serum is given. The cultures of both children as well as the three women were reported as positive. Milk extract from the three women was added to H9 cell cultures as well as lymphocyte cultures stimulated with PHA and cultivated with IL-2. The cultures were treated with polybrene and some cultures were also treated with antibody to γ-interferon. All the H9 cultures were reported to react with the rabbit antiserum. Only the lymphocytes cultured with the milk extract from the third woman reacted with the rabbit antiserum and then only when the antibody to γ-interferon was added. Supernatants from the H9 cells were added to cultures of "HUT 78 cells", "with the appearance of RT activity", which was interpreted as proving transmission of HIV from the H9 to the HUT78 cells.[293]

Comments

(a) reaction between some proteins found in cells and rabbit hyperimmune serum against HIV is not proof of viral isolation;

(b) The reaction may be considered proof for detection but if and only if there is proof the reaction is specific. No such proof exists. To the contrary even Gallo and Montagnier admit that many cellular proteins react with the "HIV" antibodies;[10,294,295]

(c) According to the authors, the immunoassays were conducted using "rabbit hyperimmune serum against HTLV-III, monoclonal antibodies directed at HTLV-III p24 and p15". However:

  (i) The p24 and p15 are said to be proteins coded by the *gag* gene of HIV;

  (ii) According to one of the best known HIV experts, William Blattner, "it may be feasible to use viral antigen probes to look for cross-reactive antibodies, since certain viral proteins may be highly conserved between subtypes of viruses";[86]

PART IV
EVIDENCE CLAIMED TO PROVE AZT AND NEVIRAPINE REDUCE MCT OF HIV

4.1 Introduction

At present, it is generally accepted that both AZT and nevirapine prevent mother to child transmission of HIV. The only way to prove that a drug inhibits mother to child transmission of HIV is to conduct prospective, randomised, double-blind controlled studies. Being double-blind ensures that bias is minimised by denying both doctor and patient all knowledge of which drug, active or placebo, the patients are receiving. The study should compare two groups of seropositive women and their infants matched in all respects in which one group of mother-child pairs receive the drug and the other the placebo. Since the tests used to prove MCT and the putative effects of the drug are non-specific, it may be necessary to introduce an additional control group of children, these being children of non-infected women who do not receive the drug.

4.2 The ACTG 076 study

4.2.1 Importance of the ACTG 076 study

In 1994 researchers from 59 centres in the USA and France published a paper entitled "Reduction of Maternal-Infant Transmission of Human Immunodeficiency Virus Type 1 with Zidovudine Treatment". This study, known as the Pediatric AIDS Clinical Trial Group 076 (ACTG 076), is the basis for all subsequent use of AZT in HIV positive, pregnant women.[331] According to David Wilkinson and James McIntyre, in their article published in the *South African Medical Journal* in 1998, "the landmark ACTG 076 trial done in the USA and France showed that a lengthy, complex and expensive regimen of zidovudine (ZDV) given through pregnancy, in labour and to the newborn reduced MTCT of HIV by 67% in women who did not breast-feed. Widespread implementation of this regimen in the USA and other countries, including France had led to a marked reduction in the incidence of paediatric AIDS–a public health triumph".[332] (In fact it is accepted that the "decrease" in mother to child transmission began well before the introduction of AZT[231]). Given the importance attached to this study, and that to date it is the only study which the authors claim as "randomised, double-blind, placebo-controlled", a thorough analysis of its data is warranted.

4.2.2 Patients and Methods used in the ACTG 076 study

In the text the authors state: "From April 1991 through December, 1993, 477 pregnant women were enrolled at 59 centers. Of the eligible women, 409 gave birth during this period to a total of 415 live-born infants, including 403 singletons and 6 sets of twins. Two women had a history of HIV seropositivity but were later found not to be infected. These two women and an infant born to one of them were excluded from the analysis. Twelve women (one of whom had a creatinine concentration outside the specified range) withdrew from the study before delivery; data on these women were included up to the time of withdrawal".

In Table 2 entitled "Characteristics of the Women and Infants in the Study" there are only 461 women, 86 white (48 treated with AZT and 38 placebo); 234 Black (107 AZT and 127 placebo); 132 Hispanic (71 AZT, 61 placebo) and 9 others (6 AZT, 3 placebo). In Table 1, "Status of Mothers and Infants in the Study, as of December 20, 1993", the authors state that of the 477 mothers enrolled they ended up with 409 "Eligible mother-infant pairs", of which only 363 were used in their statistical analysis". The racial distribution of the 363 mother-infant pairs is not mentioned. Neither in this regard are differences between the AZT and placebo groups.

Again, given that:

1. AIDS was first diagnosed in 1981 in the USA, in gay and bisexual men;
2. Up to 20% of men who consider themselves gay have sex with women;[246,247]
3. By 1982 the vast majority of haemophiliacs in the USA were infected with HIV;
4. Education in regard to safe sexual practices was introduced only in 1985-86;
5. Even by 1997, with no effort in safe sex education spared, 25% of partners of infected individuals enrolled in on going studies conducted by HIV experts did not practise safe sex;[194]
6. Women who are pregnant have not consistently practised safe sex;

it follows that there should have been no difficulties in the 1990s to recruit HIV infected mothers. Instead,

(a) The ACTG076 researchers required 59 institutions in two continents to recruit 477 mothers;

(b) Of the mothers recruited only a minority (18.6%) are white.

PART V
ALTERNATIVE PREVENTION OF THE PUTATIVE MOTHER TO CHILD TRANSMISSION OF HIV

5.1 Introduction

It makes no sense, indeed it is contrary to the Hippocratic Oath, to administer a drug that is toxic and devoid of therapeutic effects. While there is no proof that neither AZT nor nevirapine possess antiretroviral effects, (indeed, given their pharmacological properties and dosing schedules, it is not possible for these agents to have such effects, see Part IV) there is ample evidence that both drugs are toxic to adults and, in the case of AZT, to the children of mothers administered the drug during pregnancy.

The claim of a beneficial effect of AZT and nevirapine on MCT is based on their effects on test parameters. These parameters are

(i) antibody/antigen reactions, that is, the reaction of antigens present in the antibody test kits with antibodies in patient sera the antibody tests;
(ii) the reaction of antibodies to p24 with antigens present in test cultures, "HIV isolation";
(iii) the PCR test.

To date there is no evidence that these drugs favourably effect these parameters. Even if there were such proof, their benefits in patients can be judged only by effects on disease progression or mortality rates. The current vogue for the use of surrogate end points to document benefit and harm from particular treatments requires surrogates which reliably predict overall clinical outcomes. For a variety of reasons, "In practice this frequently fails…The validity of a surrogate end point has rarely been rigorously established". For example, "Predictions having an accuracy of approximately 50%, such as the accuracy seen with the CD4 count in the HIV setting, are as informative as a toss of a coin…Proper validation of surrogates also requires an in-depth understanding of the causal pathways of the disease process as well as the intervention's intended mechanisms of action. Such insights are rarely achievable…In definitive phase 3 trials, except for rare circumstances in which the validity of the surrogate end point has already been rigorously established, the primary end point should be the true clinical outcome".[369] In this regard the presently available evidence indicates that AZT may worsen rather than improve end points. (No similar data are presently available for nevirapine but the drug is toxic to adults and may be even more toxic than AZT.[370-373] Indeed in April 2000 "severe and life-threatening cutaneous and hepatic reactions" caused by nevirapine prompted the European Agency for the Evaluation of Medicinal Products to issue a public warning on the EMEA website.[374] Thus one would also expect this drug to be also toxic to infants).

5.2 The safety of AZT

Children studied in the Pediatric AIDS Clinical Trial Group 076 who received either AZT or placebo, were enrolled in "a long term observational protocol", Protocol 219. The aim was, "To evaluate the long-term effects of in utero exposure to zidovudine [AZT] vs placebo" in uninfected children. The authors concluded:

(a) "No significant short-term toxic effects were observed in PACTG 076 for those mothers and infants who received zidovudine";

(b) "No adverse effects were observed in HIV-uninfected children with in-utero and neonatal exposure to zidovudine followed up for as long as 5-6 years".

However, neither the authors of the PACTG 076 protocol[331] nor those of the PACT 219 identified their placebo. In the original 1994 publication from the PACTG 076 study the authors reported that 22 children stopped therapy "because of toxic effects (11 in each group)". Most importantly, in the 219 study, instead of following blindly all the children from the 076 study as is required in this type of epidemiological research, the authors selected only 234 (122 in the AZT group and 112 in the placebo), without giving any reason(s). At the end of the study, when the analysis was performed only "86% of the uninfected children enrolled in PACTG 219 were still participating in the study; 26 children were lost to follow-up or their caregivers refused further contact". No data are provided on the classification of the children enrolled in the 219 but lost to follow-up. Although in the PACTG 219 protocol "Echocardiograms and ophthalmology examinations (including visual acuity assessment and funduscopic examination) were required for all children by 36 months of age", only:

PART VI
GENERAL DISCUSSION

6.1 HIV Tests

In 1983 Montagnier and his colleagues claimed to have proven the existence of a new, unique retrovirus and of its constituent proteins and RNA by isolating (purifying) retroviral particles. However, no proof of purification was presented. In July 1997 Montagnier admitted that, despite their 1983 claims, he and his colleagues did not purify HIV and in fact the material which they called "purified" virus did not contain even particles "with the morphology typical of retroviruses". Also in 1997 the first electron micrographs of "purified HIV" were published establishing that "purified HIV" is particulate material consisting overwhelmingly of cellular fragments in which are interspersed a small number of particles whose morphology more resembles that of retrovirus particles than the predominant particles but none of which have all the structural features of retrovirus particles. The fact that particles similar to the latter are observed in material from "non-infected" cultures obtained in the same manner as the "purified HIV", and that this material consists of the same proteins (quantitative differences aside) as the "purified" virus, demonstrates beyond reasonable doubt that "purified HIV" is devoid of retroviral particles, proteins and RNA. Yet from this material proteins and RNA have been selected and employed as antigens in antibody tests and primers and probes in PCR and hybridisation experiments to prove humans infected with a lethal retrovirus.

If the RNA that has been arbitrarily selected from the 1.16 gm/ml band is the genome of an exogenous retrovirus, then:

(i) there must be evidence to prove the existence of a unique molecular entity "HIV RNA", and a corresponding fragment of DNA ("HIV DNA") which has a unique length and unique nucleic acid sequences;

(ii) when the full length of "HIV RNA" or "HIV DNA" is used for hybridisation studies all infected people should give a positive result. That is, if the "HIV RNA" is the genome of an exogenous virus which infects individuals who have AIDS or those at risk then the full length of this RNA (cDNA) should be present in fresh uncultured tissue from all these individuals and in nobody else.

This is not the case. From the beginning of the HIV era it became obvious that no two HIV genomes are identical, not even from the same individual.[483-487] While the genomes of the most variable RNA viruses do not differ by more than 1%[488] and the difference between the human and the chimpanzee genomes is no more than 2%, there is up to 40% variation between "HIV" genomes.[489]

Gallo and his colleagues were the first to report hybridisation studies using fresh lymphocytes from AIDS patients and those at risk. Summarising their finding they wrote: "We have previously been able to isolate HTLV-III from peripheral blood or lymph node tissue from most patients with AIDS or ARC" (they "isolated" it from approximately 50% of patients). "However, as shown herein, HTLV-III DNA is usually not detected by standard Southern Blotting hybridisation of these same tissues and, when it is, the bands are often faint…the lymph node enlargement commonly found in ARC and AIDS patients cannot be due directly to the proliferation of HTLV-III-infected cells…the absence of detectable HTLV-III sequences is Kaposi's sarcoma tissue of AIDS patients suggests that this tumor is not directly induced by infection of each tumor cell with HTLV-III…the observation that HTLV-III sequences are found rarely, if at all, in peripheral blood mononuclear cells, bone marrow, and spleen provides the first direct evidence that these tissues are not heavily or widely infected with HTLV-III in either AIDS or ARC".[490] These findings were confirmed by many other researchers. The finding that when the results were positive the hybridisation bands were "faint", "low signal" was interpreted as proof that HIV seropositive individuals contain HIV DNA in small numbers of cells and at low copy numbers, an interpretation which became generally accepted although Gallo and his colleagues had an alternative explanation. "Theoretically, this low signal intensity could also be explained by the presence of virus distantly homologous to HTLV-III in these cells".[490] By 1994 Gallo admitted "We have never found HIV DNA in the tumor cells of KS…In fact we have never found HIV DNA in T-cells".[280]

To improve detection PCR was introduced. However:

(a) "a striking feature of the results obtained" with this method, as with the standard hybridisation technique, "is the scarcity or apparent absence of viral DNA in a proportion of patients";[484]

(b) there is no proof that the PCR amplifies the "HIV RNA" (DNA).

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APPENDIX II
CDC 1987 Classification System for HIV Infection in Children under 13 years of age

Source: Classification system for human immunodeficiency virus (HIV) infection in children under 13 years of age. *Morbidity and Mortality Weekly Reports* 1987;36**:**225-30, 235-6.

Infants and children under 15 months of age with perinatal infection – infection in infants and children up to 15 months of age who were exposed to infected mothers in the perinatal period may be defined by one or more of the following:

1) the identification of the virus in blood or tissues,
2) the presence of HIV antibody as indicated by a repeatedly reactive screening test (e.g., enzyme immunoassay) plus a positive confirmatory test (e.g., Western blot, immunofluorescence assay) in an infant or child who has abnormal immunologic test results indicating both humoral and cellular immunodeficiency (increased immunoglobulin levels, depressed T4 (T-helper) absolute cell count, absolute lymphopenia, decreased T4/T8 ratio) and who meets the requirements of one or more of the subclasses listed under class P-2 (described below), or
3) the confirmation that a child's symptoms meet the previously published CDC case definition for pediatric AIDS (1,2)

Classification System

Children fulfilling the definition of HIV infection discussed above may be classified into one of two mutually exclusive classes based on the presence or absence of clinical signs and symptoms. Class Pediatric-1 (P-1) is further subcategorized on the basis of the presence or absence of immunologic abnormalities, whereas Class P-2 is subdivided by specific disease patterns. Once a child has signs and symptoms and is therefore classified in P-2, he or she should not be reassigned to class P-1 if signs and symptoms resolve.

Perinatally exposed infants and children whose infection status is indeterminate are classified into class P-0.

Class P-0. Indeterminate infection. Includes perinatally exposed infants and children up to 15 months of age who cannot be classified as definitely infected according to the above definition but who have antibody to HIV, indicating exposure to a mother who is infected.

Class P-1. Asymptomatic infection. Includes patients who meet one of the above definitions for HIV infection but who have had no previous signs or symptoms that would have led to classification in Class P-2.

Subclass A – Normal immune function. Includes children with no immune abnormalities associated with HIV infection.

Subclass B – Abnormal immune function. Includes children with one or more of the commonly observed immune abnormalities associated with HIV infection, such as hypergammaglobulinemia, T-helper (T4) lymphopenia, decreased T-helper/T-suppressor (T4/T8) ratio, and absolute lymphopenia.
Other causes of these abnormalities must be excluded.

Subclass C – Not tested. Includes children for whom no or incomplete (see above) immunologic testing has been done.

Class P-2. Symptomatic infection. Includes patients meeting the above definitions for HIV infection and having signs and symptoms of infection. Other causes of these signs and symptoms should be excluded. Subclasses are defined based on the type of signs and symptoms that are present. Patients may be classified in more than one subclass.

Subclass A – Nonspecific findings. Includes children with one or more of the unexplained nonspecific findings persisting for more than 2 months, including fever, failure-to-thrive or weight loss of more than 10% of baseline, hepatomegaly, splenomegaly, generalized lymphadenopathy (lymph nodes measuring at least 0.5 cm present in two or more sites, with bilateral lymph nodes counting as one site), parotitis, and diarrhea (three or more loose stools per day) that is either persistent or recurrent (defined as two or more episodes of diarrhea accompanied by dehydration within a 2-month period).

Subclass B – Progressive neurologic disease. Includes children with one or more of the following progressive findings: 1) loss of developmental milestones or intellectual ability, 2) impaired brain growth (acquired microcephaly and/or brain atrophy demonstrated on computerized tomographic scan or magnetic resonance imaging scan), or 3) progressive symmetrical motor deficits manifested by two or more of these findings: paresis, abnormal tone, pathologic reflexes, ataxia, or gait disturbance.

APPENDIX III
CDC 1994 Revised Classification System for HIV Infection in Children under 13 years of age

Source: 1994 Revised Classification System for Human Immunodeficiency Virus Infection in Children Less Than 13 Years of Age. *Mortality and Morbidity Weekly Reports* 1994;43 (RR-12)**:**1-10.

Summary
This revised classification system for human immunodeficiency virus (HIV) infection in children replaces the pediatric HIV classification system published in 1987 (1). This revision was prompted by additional knowledge about the progression of HIV disease among children. In the new system, infected children are classified into mutually exclusive categories according to three parameters: a) infection status, b) clinical status, and c) immunologic status. The revised classification system reflects the stage of the child's disease, establishes mutually exclusive classification categories, and balances simplicity and medical accuracy in the classification process. This document also describes revised pediatric definitions for two acquired immunodeficiency syndrome-defining conditions.

INTRODUCTION

Following the initial report in 1982 of acquired immunodeficiency syndrome (AIDS) in children (2), it became evident that the clinical characteristics of AIDS in children were different from those in adults. In 1987, CDC published a classification system for children infected with human immunodeficiency virus (HIV) (1), the causative agent of AIDS. This classification system categorized clinical manifestations of HIV infection in children based on the limited data available early in the epidemic. New knowledge about the progression of HIV disease among children warranted revision of the 1987 classification system to better reflect the disease process.

In 1991, CDC convened a working group of Public Health Service and other consult-ants to discuss revision of the pediatric HIV classification system. The 1994 revised classification system was developed through ongoing collaborations with the consult-ants following the 1991 meeting. The goal of the working group was to construct a revised system that would:

- reflect the stage of disease for an HIV-infected child (i.e., the child's placement in the classification should have prognostic significance);
- establish mutually exclusive classification categories; and
- balance simplicity and medical accuracy in the classification process.

In the new system (Table 1), HIV-infected children are classified into mutually exclusive categories according to three parameters: a) infection status, b) clinical status, and c) immunologic status. Once classified, an HIV-infected child cannot be reclassified in a less severe category even if the child's clinical or immunologic status improves.

DIAGNOSING HIV INFECTION IN CHILDREN

Diagnosis of HIV infection in children born to HIV-infected mothers (Box 1) is complicated by the presence of maternal anti-HIV IgG antibody, which crosses the placenta to the fetus. Virtually all these children are HIV-antibody positive at birth, although only 15%–30% are actually infected. In uninfected children, this antibody usually becomes undetectable by 9 months of age but occasionally remains detect-able until 18 months of age. Therefore, standard anti-HIV IgG antibody tests cannot be used to indicate reliably a child's infection status before 18 months of age (3). Polymerase chain reaction (PCR) and virus culture are probably the most sensitive and specific assays for detecting HIV infection in children born to infected mothers (4–6). Use of these assays can identify approximately 30%–50% of infected infants at birth and nearly 100% of infected infants by 3–6 months of age (7).

The standard p24-antigen assay is less sensitive than either virus culture or PCR, especially when anti-HIV antibody levels are high, because it fails to detect immune-complexed p24 antigen (8). However, modification of the p24-antigen assay to dissociate immune complexes has increased its sensitivity in diagnosing HIV infection among children exposed to HIV (9).

Other laboratory assays (e.g., anti-HIV IgA and ELISPOT/in vitro antibody production [IVAP]) have not been included in the algorithm for determining infection status because they are not commonly used. In addition, they are less sensitive than both PCR or virus culture. However, clinicians who determine a child's antiretroviral therapy on the basis of such assays may use them to classify the child as being infected.

Some children develop severe clinical conditions resulting from HIV infection before their infection status has been sufficiently established. For the purposes of classification, a child meeting the criteria for AIDS in the 1987 case definition (10) should be considered HIV-infected—even in the absence of definitive laboratory assays.

APPENDIX IV
CDC 2000 Revised AIDS Surveillance Definition

Source: Centers for Disease Control and Prevention. *Mortality and Morbidity Weekly Reports* 1999;48 (RR-13):1-27, 29-31.

Revised Surveillance Case Definition for HIV Infection*

This revised definition of HIV infection, which applies to any HIV (e.g., HIV-1 or HIV-2), is intended for public health surveillance only. It incorporates the reporting criteria for HIV infection and AIDS into a single case definition. The revised criteria for HIV infection update the definition of HIV infection implemented in 1993 (18); the revised HIV criteria apply to AIDS-defining conditions for adults (18) and children (17,19), which require laboratory evidence of HIV. This definition is **not** presented as a guide to clinical diagnosis or for other uses.

I. In adults, adolescents, or children aged ≥ 18 months † , a reportable case of HIV infection must meet at least one of the following criteria:

Laboratory Criteria

- Positive result on a screening test for HIV antibody (e.g., repeatedly reactive enzyme immunoassay), followed by a positive result on a confirmatory (sensitive and more specific) test for HIV antibody (e.g., Western blot or immunofluorescence antibody test)

or

- Positive result or report of a detectable quantity on any of the following HIV virologic (nonantibody) tests:
  - HIV nucleic acid (DNA or RNA) detection (e.g., DNA polymerase chain reaction [PCR] or plasma HIV-1 RNA) §
  - HIV p24 antigen test, including neutralization assay
  - HIV isolation (viral culture)

OR

Clinical or Other Criteria (if the above laboratory criteria are not met)

- Diagnosis of HIV infection, based on the laboratory criteria above, that is documented in a medical record by a physician

or

Conditions that meet criteria included in the case definition for AIDS

II. In a child aged <18 months, a reportable case of HIV infection must meet at least one of the following criteria:

Laboratory Criteria

Definitive

- Positive results on two separate specimens (excluding cord blood) using one or more of the following HIV virologic (nonantibody) tests:
  - HIV nucleic acid (DNA or RNA) detection
  - HIV p24 antigen test, including neutralization assay, in a child **≥** 1 month of age
  - HIV isolation (viral culture)

or

---

*Draft revised surveillance criteria for HIV infection were approved and recommended by the membership of the Council of State and Territorial Epidemiologists (CSTE) at the 1998 annual meeting (11). Draft versions of these criteria were previously reviewed by state HIV/AIDS surveillance staffs, CDC, CSTE, and laboratory experts. In addition, the pediatric criteria were reviewed by an expert panel of consultants. [External Pediatric Consultants: C. Hanson, M. Kaiser, S. Paul, G. Scott, and P. Thomas. CDC staff: J. Bertolli, K. Dominguez, M. Kalish, M.L. Lindegren, M. Rogers, C. Schable, R.J. Simonds, and J. Ward]

† Children aged ≥ 18 months but <13 years are categorized as "not infected with HIV" if they meet the criteria in **III.**

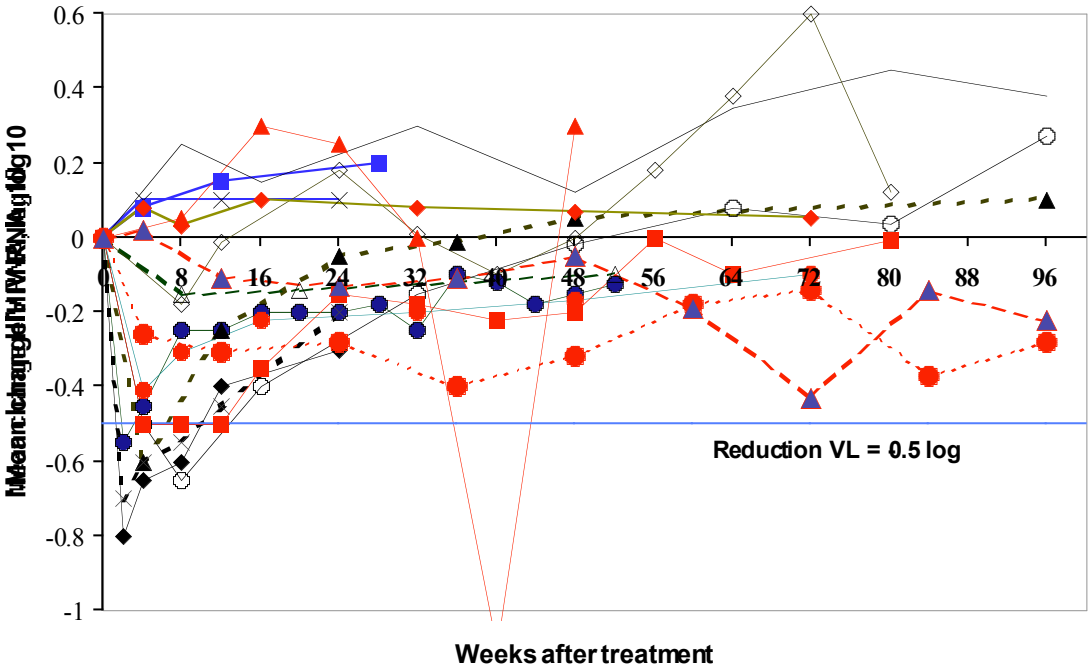
APPENDIX V
Reported Literature Measuring AZT triphosphorylation in Humans

In none of these studies does AZTTP reach the concentration estimated ideally *in vitro* of 0.7 μM

Year	Peak Concentration of Triphosphorylated AZT Reported	Reference
1991	0.5 pmol/10^6 cells	Kuster H, et al. J Infect Dis; 164: 773–776
1991	56 pmol/10^7 cells (5.6 pmol/10^6 cells)	Toyoshima T, et al. Analytical Bioch; 196: 302–307
1992	0.14 pmol/10^6 cells	Slusher JT, et al. Antimic Agents & Chemoth; 36: 2473–2477
1994	326 fmol/10^6 cells (0.326 pmol/10^6 cells)	Robbins BL, et al. Antimicrob Agents Chemother; 38: 115–121
1994	0.06 pmol/10^6 cells	Barry MG, et al. AIDS; 8: F1–F5
1996	95 fmol/10^6 cells (0.095 pmol/10^6 cells)	Rodman JH, et al. J Infec Dis; 174: 490-499
1996	0.069 pmol/10^6 cells	Peter K, et al. J Pharm & Biomed Anal; 14: 491–499
1996	0.042 pmol/10^6 cells (average)	Peter K and Gambertoglio JC. Clin Pharmacol Ther; 60: 168–176
1996	0.07 pmol/10^6 cells	Barry MG, et al. AIDS; 10: 1361–1367
1998	0.046 pmol/10^6 cells, in mononuclear cells from lymph nodes. 0.085 pmol/10^6 cells in PBMC	Peter K et al. AIDS; 12: 1729–1731[a]
1998	0.07 pmol/10^6 cells	Robbins BL, et al. Antimicrob Agents Chemother; 42: 2656-2660
1998	160 fmol/10^6 cells (average) (0.16 pmol/10^6 cells)	Fletcher CV, et al. Clin Pharmacol Ther 64: 331–338
1999	329 fmol/10^6 cells (0.329 pmol/10^6 cells)	Rodman JH et al. J Infec Dis; 180:1844-50
1999	193 fmol/10^6 cells (0.193 pmol/10^6 cells)	Font E, et al. Antimicrob Agents Chemother; 43: 2964-8
2000	0.32 pmol/10^6 cells	Wattanagoon Y, et al. Antimicrob Agents Chemother; 44: 1986-1989

1 μmol = 10^{-6} mole; 1 pmol = 10^{-12} mole; 1 fmol = 10^{-15} mole; 1 pmol/10^6 cells ≈ 1 μM

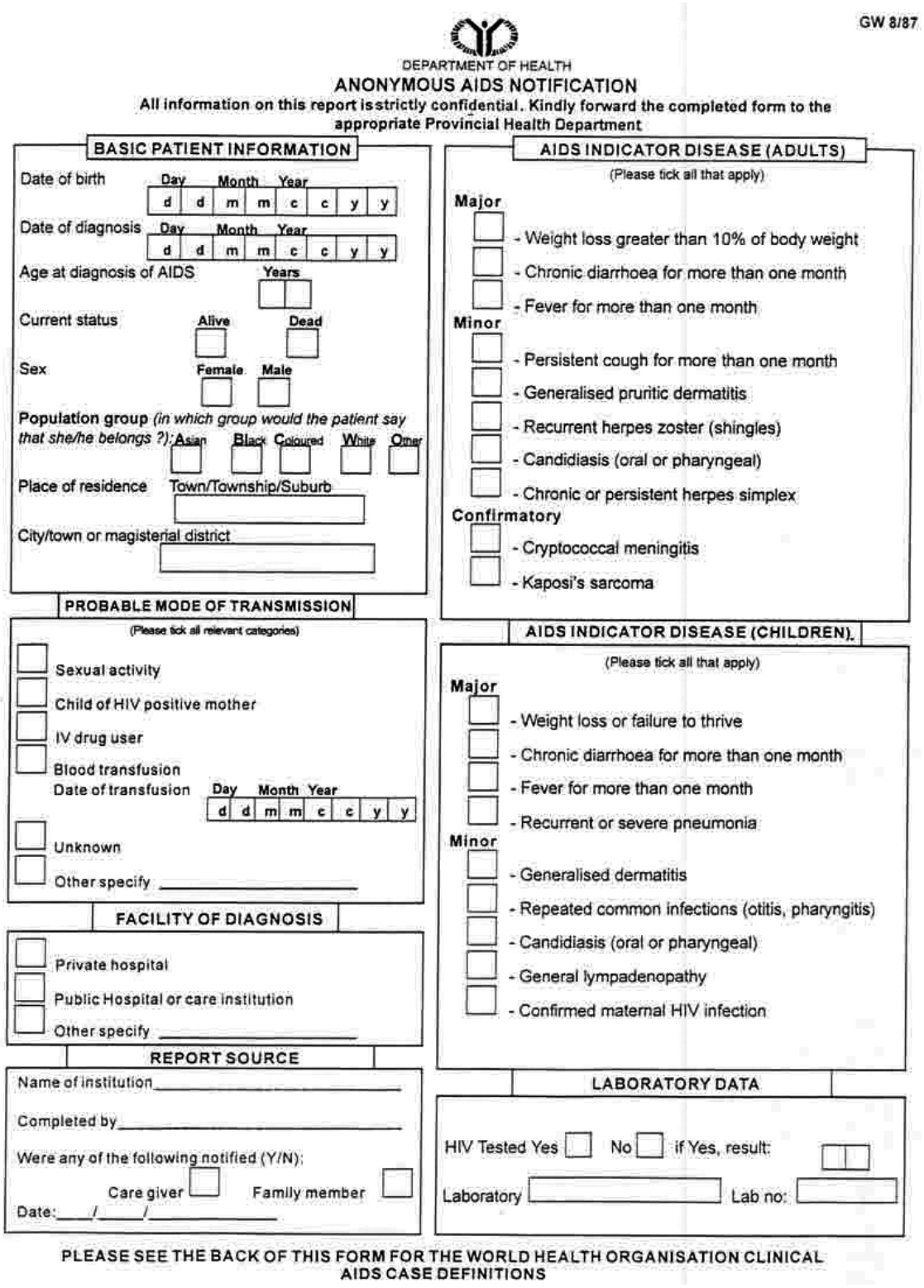
APPENDIX VI
AZT administration versus "Viral Load"



a)	Eron JJ et al. NEJM 1995;333:1662-9	
b)	De Jong MD, et al. PNAS 1996;93:5501-6	
c)	Katlama C, et al. JAMA 1996;276:118-25	
d)	Katlama C, et al. JAMA 1996;276:118-25	
e)	Staszewski S et al. JAMA 1996;276:111-7	
f)	Carr A, AIDS 1996;10:635-41	
g)	O'Brien WA, et al. NEJM 1996;334;426-31	
h)	O'Brien WA, et al. NEJM 1996;334;426-31	
i)	Katzenstein D, et al. NEJM 1996:1091-8	
j)	Bakshi SS, et al. J Infect Dis 1997;175:1039-50	
k)	Bruisten SM et al. AIDS Res & Hum Retr 1998;12:1053-8	
l)	Delta Committee. AIDS, 1999:57-65	
m)	Delta Committee. AIDS, 1999:57-65	
n)	Lillo FB, et al. AIDS 1999;13:791-6	
o)	Arch Dis Child 2001: 84: 230-60	
p)	Arch Dis Child 2001: 84: 230-60	

APPENDIX VII
AIDS Reporting form for South Africa

Department of Health, Pretoria.



GW 8/87

DEPARTMENT OF HEALTH

ANONYMOUS AIDS NOTIFICATION

All information on this report is strictly confidential. Kindly forward the completed form to the appropriate Provincial Health Department

BASIC PATIENT INFORMATION

Date of birth Day Month Year (d d m m c c y y)

Date of diagnosis Day Month Year (d d m m c c y y)

Age at diagnosis of AIDS Years

Current status Alive Dead

Sex Female Male

Population group *(in which group would the patient say that she/he belongs ?)*: Asian Black Coloured White Other

Place of residence Town/Township/Suburb

City/town or magisterial district

PROBABLE MODE OF TRANSMISSION

(Please tick all relevant categories)

- Sexual activity
- Child of HIV positive mother
- IV drug user
- Blood transfusion
  Date of transfusion Day Month Year (d d m m c c y y)
- Unknown
- Other specify _____

FACILITY OF DIAGNOSIS

- Private hospital
- Public Hospital or care institution
- Other specify _____

REPORT SOURCE

Name of institution_____

Completed by_____

Were any of the following notified (Y/N):

Care giver Family member

Date:____/____/_____

AIDS INDICATOR DISEASE (ADULTS)

(Please tick all that apply)

Major

- Weight loss greater than 10% of body weight
- Chronic diarrhoea for more than one month
- Fever for more than one month

Minor

- Persistent cough for more than one month
- Generalised pruritic dermatitis
- Recurrent herpes zoster (shingles)
- Candidiasis (oral or pharyngeal)
- Chronic or persistent herpes simplex

Confirmatory

- Cryptococcal meningitis
- Kaposi's sarcoma

AIDS INDICATOR DISEASE (CHILDREN)

(Please tick all that apply)

Major

- Weight loss or failure to thrive
- Chronic diarrhoea for more than one month
- Fever for more than one month
- Recurrent or severe pneumonia

Minor

- Generalised dermatitis
- Repeated common infections (otitis, pharyngitis)
- Candidiasis (oral or pharyngeal)
- General lympadenopathy
- Confirmed maternal HIV infection

LABORATORY DATA

HIV Tested Yes No if Yes, result:

Laboratory Lab no:

PLEASE SEE THE BACK OF THIS FORM FOR THE WORLD HEALTH ORGANISATION CLINICAL AIDS CASE DEFINITIONS

APPENDIX VIII
AIDS Reporting form for Uganda

MINISTRY OF HEALTH/ACP
P.O.BOX 8, ENTEBBE
TEL: 20353, 20534

Rev.09/91

UGANDA MINISTRY OF HEALTH
ADULT (12 years and above) AIDS REPORTING FORM

Instructions:
Please fill out this form for every patient diagnosed with AIDS at the initial time of diagnosis.
Diagnosis will be based on the Uganda WHO modified clinical case definition

FORM NO:

HOSPITAL_____

HOSPITAL REGISTER NO:_____ AGE_____ SEX_____

DISTRICT OF RESIDENCE_____ OCCUPATION_____

DOES THE PATIENT HAVE THE FOLLOWING?

SYMPTOMS/PHYSICAL FINDINGS. (PLEASE TICK)

☐ DISSEMINATED KAPOSI SARCOMA

☐ CRYPTOCOCCAL MENINGITIS

MAJOR SIGNS

☐ WEIGHT LOSS AT LEAST 10%

☐ DIARRHOEA AT LEAST 1 MONTH

☐ FEVER AT LEAST 1 MONTH

MINOR SIGNS

☐ ORO-PHARYNGEAL CANDIASIS

☐ PRURITIC SKIN RASH

☐ HERPES ZOSTER

☐ GENERALISED LYMPHADENOPATHY

☐ COUGH AT LEAST 1 MONTH (WITHOUT TB)

☐ CHRONIC ULCERATED HERPES SIMPLEX

☐ TUBERCULOSIS

☐ OTHERS.....

DATE____/____/____ NAME OF REPORTING OFFICER.....

PLEASE RETURN THE COMPLETED FORMS TO ACP/MINISTRY OF HEALTH

APPENDIX IX
Calculation of the probability of HIV transmission between sexual partners

Livio Mina. Mathematician and Statistician, Department of Medical Physics, Royal Perth Hospital, Perth, Western Australia

Suppose that for each contact episode there is a constant probability, p of being infected which is independent of any previous contact history.

The number of contacts needed to first contract the disease follows the geometric distribution with probability function

$$P(n) = p\,(1-p)^{n-1}$$

where P(n) is the probability of first contracting the disease at the nth contact.

If we are interested in knowing the probability of having caught the disease after a given number of contacts (say n) we must sum all the probabilities of first catching the disease at the first, second, third, etc. contact up to n. This is somewhat tedious, and for this question we can turn instead to the Binomial distribution which gives us the distribution of the number of times we would catch the disease (at least notionally) in n contact episodes.

The probability function here is

$$P(x) = n!/[x!(n-x)!]\;\; p^x\,(1-p)^{n-x}$$

where P(x) is the probability of being infected x times (sic) in n contacts.

The idea of multiple infection may not make a great deal of sense biologically but we can legitimately ask what is the probability that there be no infection at all (ie. x = 0) after n contacts. From the formula we see that this will be $(1-p)^n$ so that the probability of contracting the disease (regardless of the notion of multiple infections) is $1 - (1-p)^n$.

APPENDIX X
Email Correspondence with the CDC re Scientific Basis of HIV Testing in Children and Adults

March 14th 2001 V. Turner to Helene Gayle

Dear Helene,

I would be most grateful if you could explain the scientific basis for the following:

According to the latest CDC AIDS surveillance definition,

"In adults, adolescents, and children infected by other than perinatal exposure, plasma viral RNA nucleic acid tests should **NOT** be used in lieu of licensed HIV screening tests (e.g., repeatedly reactive enzyme immunoassay)".

Is not the basis of these tests the existence and recognition of nucleic acid sequences unique to HIV? Is it not true that by this means HIV specific primers/probes detect and count nucleic acid molecules which are HIV and none other? If these tests respond to HIV specific sequences how can they fail to diagnose HIV infection? Is the CDC suggesting that these tests should "**NOT**" be used because they also detect and measure nucleic acid sequences which are not HIV? Regardless, if the tests are not *bona fide* in adults, why are they permitted in infants < 18 months of age?

On the other hand, if the nucleic acid tests can be used to diagnose humans <18 months of age, then why not humans >18 months of age? Certainly the antibody tests are problematic before 18 months of age but that does not provide an answer to the question.

Best wishes,

Val Turner MD

March 22nd 2001 Helene Gayle declines to answer but refers V Turner's email to Dr. ML Lindegren.

Dr. Turner,

I am following up on an email requesting information on the CDC HIV case definition from Dr. Gayle. I would love to be able to help, can you forward to me your questions on the use of the RNA assays for children,

Best,

Mary Lou Lindegren

March 22nd 2001

Reply to Dr. Lindegren

Dear Dr. Lindergren,

Thank you very much for your kind offer to answer some questions in relation to the CDC 2000 Revision AIDS definition. Please let me first explain that we are researching matters arising out of the Presidential AIDS Panel meeting which met last July in Johannesberg and which Helene attended.

According to the CDC 2000 AIDS surveillance definition,

"In adults, adolescents, and children infected by other than perinatal exposure, plasma viral RNA nucleic acid tests should **NOT** be used in lieu of licensed HIV screening tests (e.g., repeatedly reactive enzyme immunoassay)".

APPENDIX XI
A Critical Examination of the Evidence for the Existence of HIV

INTRODUCTION

Following the appearance of AIDS in 1981 many aetiological factors were proposed. In May 1983 Montagnier announced the discovery of a retrovirus, now known as HIV, from lymphatic tissue of a gay man with lymphadenopathy. One year later Gallo reported data which "suggests that HTLV-III [HIV] is the primary cause of AIDS". By 1986 the scientific community accepted the Gallo assertion that the same data was "clearcut evidence" that HIV is the causative agent of the clinical syndrome. Even today the five *Science* papers published by these French and American groups are still widely regarded as proving beyond all reasonable doubt that HIV exists and is the cause of AIDS.

However, not all scientists accepted these findings. In 1987 Peter Duesberg published an invited paper in *Cancer Research* on retroviruses and cancer in which he also questioned the role of HIV in AIDS.[1] At the same time one of us (EPE) also challenged the theory[2] including the data claimed to prove the existence of HIV. Papadopulos-Eleopulos also proposed an alternative, non-infectious aetiology and treatments based on this hypothesis. Since then our group has published papers addressing every facet of the HIV theory[2-18] including a detailed examination of HIV isolation and the HIV genome.[9,19] Here we confine ourselves largely to addressing the data published by Montagnier and Gallo in their 1983/84 *Science* papers. Genomic data are only briefly discussed because the existence of HIV and the HIV theory of AIDS were universally accepted before such data were available. For a comprehensive discussion on genomic data the reader is referred to reference 19.

RETROVIRUSES AND THEIR IDENTIFICATION

A virus possesses two characteristic properties. The first is anatomical, that is, being a microscopic particle of individual morphology, the second, the ability to generate identical progeny by synthetic processes obligatorily occurring within living cells. It is the latter attribute which defines a particle with the appearances of a virus, that is, a viral-like particle, as infectious and thus a virus. The three subfamilies (*0ncovirinae*, *Lentivirinae* and *Spumavirinae*) of *Retroviridae* (Retroviruses) are "enveloped viruses with a diameter of 100-120 nm budding at cellular membranes. Cell released virions contain condensed inner bodies (cores) and are studded with projections (spikes, knobs)".[20] The retroviral particles contain RNA and the enzyme reverse transcriptase (RT), an RNA dependent DNA polymerase which catalyses the synthesis of DNA contrary to the central dogma of biology, that is, in a direction "reverse" from DNA to RNA. According to retrovirologists, such DNA is then integrated into existing cellular DNA as a "provirus". Retroviral particles share the property of concentrating (banding) at a density of 1.16 gm/ml when centrifuged at high speeds in sucrose density gradients, a fact long used in their purification.[21,22]

All retrovirologists agree that to prove the existence of a new retrovirus one must isolate it. However, the term "virus isolation" is beset with semantic difficulties and ambiguities. The dictionary meaning of "isolation" derives from the Latin *insulatus* (made into an island) and refers to the act of separating an object from all other matter that is not that object. "Purification" means to obtain something free from impurities. In this context isolation is the same as purification. Because virus particles are small it is not possible to obtain a single, isolated particle. The next best thing is to obtain a mass of particles separate from everything else. Until the early 1980s, for the isolation of animal retroviruses as well as the "first" human retrovirus HL23V, by isolation retrovirologists meant purification. On the other hand, nowadays both basic and specialised texts rarely define "isolation" and when they do such attempts are non-illuminating. For example, Levy defines isolation as a "sample of a virus from a defined source",[23] and White as the ability to "identify a totally unforeseen virus, or even discover an entirely new agent".[24] Encompassed as "virus isolation" are listed methods of culturing specimens in tissue and chick embryo cells, as well as live animals, following by documentation therein of cytopathic and pathological effects, haemoabsorption, immunofluorescence, antigen/antibody reactions and "characterisation of the viral genome".[24-27] HIV experts, including Luc Montagnier and Robin Weiss define "virus isolation" as "propagating them [viruses] in cells in culture".[28,29] However, if "virus isolation" is to "take a sample of a virus from a defined source", or "propagating them in cells in culture", then first one must have prior proof that a virus exists in "a defined source" or "in cells in culture". One cannot know that a virus exists or define its constituents without purification (isolation) of the putative viral particles.

There are several reasons why this is mandatory: