CHAPTER 1

1.0. Background

The introduction of combined antiretroviral therapy has led to improved outcomes in HIV infected infants and young children worldwide. Treatment is based on different guidelines such as the World Health Organization (WHO) for resource poor countries, Centre for Disease Control and prevention (CDC) used in the United States of America (USA) and European criteria in European countries.

In sub-Sahara Africa, countries including Zambia have adopted the WHO Antiretroviral Therapy (ART) guidelines when initiating Highly Active Antiretroviral Therapy (HAART). The guidelines are based on studies done in the developed countries and are modified to suit the different economic backgrounds.[1, 2, 3]

According to the WHO guidelines of 2006, HIV infected infants are commenced on HAART when the clinical disease is either WHO stage 3 or 4 regardless of the CD4% or if CD4% is less than 25% in those with clinical stage 1 and 2 disease. In situations where access to virologic testing is not available, WHO encourages presumptive clinical diagnosis of severe HIV disease in children less than 18 months for initiation of HAART but to have the status confirmed as soon as possible.

Recent recommendations of 2008 are that all HIV infected infants be started on HAART regardless of immunological, virologic and clinical stage. If no virologic testing is available, clinical presumptive diagnosis should still be made for initiation of HAART. The new recommendations are based on studies that have shown improved survival for HIV infected infants started on HAART as long as they had a positive DNA PCR, as compared to those started based on deteriorating CD4%. [4-6]

According to WHO guidelines, presumptive diagnosis should be made if;

- The infant is confirmed HIV antibody positive;
- and the diagnosis of any AIDS- indicator condition can be made,
- or the infant is symptomatic with two or more of the following {defined by IMCI guidelines for severe infection (Annex A and B)};

  -Oral thrush

  -Severe pneumonia

  -Severe sepsis

Other factors that support the diagnosis of severe HIV disease in an HIV seropositive infant include;

- Recent HIV related maternal death or advanced HIV disease in the mother
- CD4% <25% in children < 12 months old
- CD4 %< 20% in children < 18 months but >12 months old

The adapted IMCI guidelines for referral of children suspected to have HIV in Zambian children aged less than five years include; seropositive mother and child and three (3) or more of the following, chronic Otitis media, low weight for age or growth faltering or history of weight loss, recurrent pneumonia, persistent diarrhea, lymphadenopathy in more than two sites (neck, axilla or groin), oral thrush, parotid enlargement for more than 14 days and health problems in sibling or other parent.[7]

Confirmation of the diagnosis of HIV infection should be sought as soon as possible in both guidelines. However, clinical algorithms are rarely more than 70% sensitive for accurate diagnosis of infection and vary considerably with age. They are less reliable in particular in children aged less than 12 months and the diagnosis being based on signs and symptoms means the HIV infection could already be advanced. They however, may still be useful in commencing potentially life saving HAART in a seriously ill child.[8, 9]

There are insufficient data available to make firm recommendations for the use of clinical algorithms combined with measurement of CD4 or other parameters to establish HIV infection. Further, very few studies have been done to validate the WHO presumptive diagnostic criteria.[8, 9]

WHO therefore encourages researchers to validate approaches to presumptive clinical diagnosis in children less than 18 months, including studies to determine if CD4 combined with clinical signs and symptoms improves the diagnosis of HIV infection for the purpose of commencing HAART.

Generally, decision making about starting HAART is particularly important for infants as the probability of death in the absence of treatment is about 30% by age 1 year and 50% by the second birthday.[10, 11]

Like in most of Southern Africa, ARVs have become readily available in Zambian health care facilities with most of the 72 districts in the country offering treatment to both adults and children. Access to ARVs in Zambia has increased from 3,500 (September, 2006) to 13,000 (December, 2007) of children started on HAART but the challenge to reach even the remote parts of Zambia with no easy access to laboratory facilities still remains. [12]

This study therefore seeks to evaluate the sensitivity of the WHO clinical algorithm for presumptive diagnosis of HIV infection and will also evaluate if use of other parameters (both clinical and CD4%) improve the sensitivity of the criteria, in HIV infants and young children who are later confirmed positive by DNA PCR.

1.1. Statement of the Problem

The 2008 WHO guidelines propose early initiation of HAART in confirmed HIV (DNA PCR) positive infants regardless of immunological and clinical criteria. However, in resource poor areas where DNA PCR facilities are unavailable, the 2008 WHO guidelines recommend use of clinical algorithms to presumptively diagnose HIV. Notwithstanding, clinical algorithms for presumptive diagnosis of HIV are rarely more than 70% sensitive for accurate diagnosis of infection and vary considerably with age. They are less reliable in particular in children aged less than 12 months.[8, 9]

Few studies have been done to validate the WHO presumptive diagnostic criteria. There are also insufficient data available to make firm recommendations of the use of clinical algorithms combined with measurement of CD4% or other parameters to establish HIV infection in infants and young children.

1.2. Study Justification

Infant diagnosis for the purpose of initiating therapy in HIV infected children remains a challenge in Zambia due to the scarcity of virological diagnostic tests outside tertiary and second level hospitals. In Zambia, almost all the 72 districts have CD 4% measuring equipment. There is thus a need to find out whether the more widely available CD4% may improve the sensitivity of the Presumptive Diagnosis of HIV in children less than 18 months to enable early commencement of HAART in the absence of DNA PCR.

1.3. Hypothesis

The W.H.O clinical presumptive diagnostic criteria will miss a number of HIV infected infants and young children requiring early treatment in cases where a definitive diagnosis of HIV (DNA PCR) is not available. Additional clinical signs and use of CD4% will increase the sensitivity.

1.4. Study Question

What is the sensitivity of the WHO criteria for presumptive diagnosis in determining HIV infection status in children <18 months?

1.5.0. Objectives

1.5.1. Main Objective

To evaluate the sensitivity of the WHO presumptive diagnostic criteria in HIV exposed children < 18 months admitted to the Department of Paediatrics and Child Health of the University Teaching hospital.

1.5.2. Specific Objectives

1.5.2.1. To determine the prevalence of HIV exposed infants and young children meeting the WHO presumptive diagnostic criteria.

1.5.2.2. To determine the proportion of these children who test positive by DNA PCR.

1.5.2.3. To determine additional clinical conditions that can improve the sensitivity of the WHO presumptive diagnostic criteria

1.5.2.4. To determine the age at presentation with signs and symptoms of presumed HIV disease

1.5.2.5. To determine if CD4% combined with signs improves the sensitivity of the presumptive diagnosis of HIV in HIV exposed infants and children.

CHAPTER 2

2.0. Literature Review

2.1. Epidemiology of HIV

HIV is one of the major causes of morbidity and mortality in the world. Of the six billion world inhabitants, UNAIDS estimates that 38 million people were living with HIV in 2007 of whom 90% are in sub-Sahara Africa and South East Asia. Children have not been spared. An estimated 2.3 million HIV infected children younger than 15 years live in sub-Sahara Africa. Southern Africa, in which Zambia belongs, accounts for one third of all global AIDS deaths.[13-15]

It is estimated that about 28,000 infants in Zambia are born with HIV infection annually. The current HIV infection rate in Zambian adults aged 15-49 years is 14.3%. Lusaka district has the largest number of people living with HIV/AIDS, as well as the largest estimate of AIDS related deaths in the country.[16]

At the University Teaching Hospital Paediatric and Child Health department, 33% of the children admitted in 2006-2007 were HIV exposed or infected with 60% of these being infants.[17] The HIV rates are higher in children admitted with tuberculosis (70%), severe malnutrition (50%), severe pneumonia and diarrhoeal disease.[18]

2.2. Transmission

Mother-to-child transmission (MTCT) of Human Immunodeficiency Virus (HIV) -1 is estimated to be the cause of at least 90% of paediatric HIV infections, with more than 700,000 children newly infected in 2006 worldwide.[19] In Zambia, more than 20,000 babies contract HIV via maternal-child transmission of HIV.[20]

Transmission occurs mainly during pregnancy, labour and delivery as well as during breastfeeding. Without intervention, the risk of transmission is 15–30% in the non-breastfed populations and 20–45% in the breastfeeding population.[19]

In Zambia majority of the women choose to breastfeed and about 40% of HIV infected mothers will transmit the infection to their baby if no intervention is made.[20]

Interventions to reduce transmission include antiretroviral (ARV) prophylaxis given to women during pregnancy and labour and to the infant in the first weeks of life. Obstetrical interventions including elective caesarean delivery, avoiding of assisted delivery such as forceps or vacuum delivery or indeed artificial rupture of membranes unless medically indicated and complete avoidance of breastfeeding.[21-23] With these interventions, new HIV infections in children are becoming increasingly rare in many parts of the world, particularly in high-income countries (less than 2%).

In developing countries, due to resource constraints, elective caesarean delivery is seldom feasible and it is often neither acceptable nor safe for mothers to refrain from breastfeeding mainly due to cultural and socio-economic reasons. In these settings, efforts to prevent HIV infection in infants initially focused on reducing transmission around the time of labour and delivery, which accounts for most of the transmission, independent of whether the mother breastfeeds or not.[24]

In a study to find out the clinical outcomes and CD4 response in children receiving HAART in primary care settings in Zambia, it was noted that despite the scale up of perinatal prevention, many children were already infected with HIV and would die in the first 90 days of ART.[25] Recently (2007), in order to increase the effectiveness of Prevention of Mother to Child Transmission (PMTCT), Ministry of Health (M.O.H) in Zambia, and other PMTCT programmes elsewhere have adopted more efficacious ARV regimens, beginning from 28 weeks of pregnancy, which can reduce the risk of transmission during pregnancy and childbirth to 2–4%.[26,27] Previously in Zambia, a single dose of NVP was used in labour and a single dose was given to the baby within the first 72 hours. Currently, AZT is commenced at 28 weeks and 3TC and NVP are given in labour. The new born baby is given a single dose of NVP and AZT is given for a week's duration. The alternative is that HAART can be commenced in eligible HIV positive pregnant women.

Despite the use of these regimens, infants still remain at substantial risk of acquiring HIV infection through breastfeeding. Research is therefore ongoing to evaluate new approaches to prevention of breast milk transmission of HIV.[28] Further, the PMTCT programme in Zambia only reached 39% of all pregnant women in need of PMTCT in 2008.

2.3. Diagnosis of HIV in children <18 months

Definitive diagnosis of HIV in infants and children younger than 18 months can only be done by conducting virologic testing. Antibody tests are of limited use as these children may still be carrying antibodies from their mothers reflecting that the child is exposed to HIV. Antibody tests are therefore used to determine the exposure status of these children.[29] Virologic tests detect the virus itself or components of the virus and therefore infection. Examples of these tests include HIV DNA PCR, RNA and P24 antigen assays.

In situations where access to virologic testing is not available but a child has signs and symptoms suggestive of HIV infection, WHO recommends making a presumptive clinical diagnosis of severe HIV infection for initiation of HAART.

There are very few centers that can do early infant Diagnosis (EID) using DNA PCR for HIV in Zambian infants and these include one DNA PCR equipment on the Copperbelt province and two in Lusaka province (one of which is at UTH) to cater for all nine provinces in Zambia.

At UTH, infants and young children <18 months are screened with a rapid HIV antibody test (determine) to confirm or exclude exposure. Positive results are followed-up with collection of a Dry Blood Spot (DBS) for DNA PCR done at contact on admission regardless of age for confirmation of HIV infection.

2.4. Presumptive diagnosis of HIV infection

Most of the data for formulation of the presumptive diagnostic criteria of HIV in children <18 months is derived from studies done in the period before antiretroviral drugs were readily available to most countries especially developing countries. Similarly in the United States of America, guidelines for clinical staging of HIV-1 disease were developed in the early years of the epidemic.[30-32] Subsequently, new definitions of HIV-1 infected children were published including clinical diagnosis of HIV-1 infection in children <18 months who meet the criteria for diagnosis of AIDS based on the 1987 surveillance case definition.[30-37] However, dependence on clinical signs and symptoms for diagnosis of HIV infection has been largely superseded in the United States and similar settings because of the availability of laboratory based methods for making a definitive diagnosis of HIV-1 infection.

In the era before antiretroviral drugs and PMTCT were readily available, infants used to present with rapidly progressive and aggressive HIV/AIDS with symptomatic cases dying within three to four weeks of the onset of symptoms as demonstrated by a prospective study done on 108 neonates in Lusaka, Zambia in which symptomatic seropositive babies presented with failure to thrive, fever, persistent or recurrent thrush, severe sepsis and hepatomegaly. Diarrhea, sepsis and haemolytic anemia were associated with death.[38] Predictors of mortality in HIV -1 infected children noted from a Kenyan study were early acquisition of HIV, high viral load, early CD4 depletion and early manifestation of HIV related symptoms such as growth retardation, neuro-developmental delay, hepatomegaly, splenomegaly and lymphadenopathy.[39] Furthermore, another Zambian study done in 2006 to determine the survival of HIV-1 infected Zambian children older than twelve months and that had never been on ART, found that malnutrition and hospitalizations for respiratory bacterial infections predicted mortality independent of immunosuppression, suggesting that they capture HIV and non-HIV related mortality where as oral candidiasis was a proxy for immunosuppression.[40]

It has been found that the diagnosis of HIV-1 infection among HIV exposed children <18 months old and living in a high HIV-prevalence resource poor setting is a challenge, as current recommended algorithms have low sensitivity, thus underscoring the need for rapid scale-up of viral assays for early infant diagnosis.[41]

In the late 1980s, pre-definitive diagnosis era, the WHO developed clinical case definitions and clinical staging systems for HIV-1 infections.[42-44]

In addition, the WHO and the United Nations Children's Fund developed "Integrated Management of Childhood Illnesses" (IMCI) strategy which provided guidelines to help diagnose and treat sick children, including those with HIV, at primary health care level.[7] Upon evaluation however, the clinical case definitions and staging systems for the diagnosis of HIV-1 infection especially for young infants in Sub-Saharan Africa were noted to be of limited sensitivity. [36, 37]

A study done in Kigali, Rwanda in 1989 to test the WHO clinical case definition in 221 consecutively hospitalized children, median age 18 months, found that despite being highly specific, the WHO case definition had low sensitivity. From this study, it was concluded that a case definition of HIV should be suspected in a child presenting with either one or both clinical signs of respiratory distress secondary to lower respiratory tract infection and generalized lymphadenopathy. Recommendations were made to test this case definition on a larger scale.[36]

In 2002, children attending a district hospital in South Africa were assessed using the HIV algorithm by a paediatrician. The validity of the algorithm in determining symptomatic HIV was compared both to the clinical diagnosis by the paediatrician and to the laboratory HIV result. The paediatrician was able to identify more infected patients than the algorithm. Results of this study were adopted by WHO to be used by countries with a high HIV prevalence to enable IMCI practitioners identify and care for HIV infected children.[8]

With information obtained from various studies in Africa, WHO in 2006 released revised case definitions of HIV infection for surveillance purposes and a revised clinical staging classification of HIV related disease in children and adults.[33] Included in these guidelines are clinical criteria for the presumptive diagnosis of severe HIV-1 disease to allow for early initiation of HAART.

2.5. Recent developments

Studies in developed countries have demonstrated decreased morbidity and mortality with early initiation of HAART suggesting that HIV infected infants who are treated before the age of three months control their HIV infection better than infants whose HAART started latter than three months of age.[5,6]

Similar findings were noted in the CHER clinical trial conducted in South Africa whose results showed that mortality decreased by 75% in HIV infected infants commenced on HAART in the first three months of life as compared to those who were started on treatment based on declining clinical and immunological progression.[4] Based on these findings, the study group has recommended modification of the 2006 WHO criteria for starting ARVS in infants (WHO 2008). Despite the changes in the trends supported by a number of studies, implementation may take some time in areas where logistics of achieving definitive diagnosis for all perinatally exposed infants are not in place. As such, clinicians may have to depend on presumptive diagnosis of HIV infection.

A study to evaluate and consolidate the WHO presumptive diagnostic criteria for starting HAART would provide a more sensitive diagnostic criteria ensuring a wider coverage of HIV infected children <18 months.

CHAPTER 3

3.0. Methodology

3.1. Study design

This was a descriptive cross sectional study of children (0-18 months) recruited over a period of 6 months at UTH.

3.2. Study Site

The study was done from the University Teaching Hospital (UTH) Department of Paediatrics and Child Health.

The UTH is the largest referral hospital for urban and peri-urban areas in Lusaka. There are 450 paediatric inpatient beds and one overnight admission ward, three general wards and specialty wards for malnutrition, isolation, diarrhea, neonatal and paediatric intensive care units.

Ill children presenting to hospital are registered by the clerk at the reception and are seen by doctors stationed in the emergency ward. The children are then sent to the overnight admission ward where they are later reviewed by senior doctors on-call before they are transferred to in-laying wards.

All infants and children admitted to the paediatric admission ward are offered provider initiated Testing and Counseling (PITC) for HIV by trained counselors as part of routine care. The rapid tests used (according to the national algorithm) are Abbott Determine for screening and Unigold for confirming of a sero positive result and this is done in both the child and the mother if she did not opt out. The rapid HIV testing is only done from Monday to Friday when counselors, Paediatric Centre of Excellence (PCOE) nurses and laboratory staff are available to do the counseling and rapid HIV testing, linkage to care and process laboratory specimen respectively.

Those that test positive and are less than 18 months old have their blood collected as a DBS for DNA PCR testing to confirm the HIV status. The blood collection is done by the (PCOE) nurses. Patients are either sent to the PCOE nurses' linkage room for enrolment into ART care or are followed up in certain wards by the PCOE nurses in cases where the patients cannot be moved for various reasons. These wards include the malnutrition ward where patients need warmth and feeding every so often as well as the isolation ward where patients with contagious conditions are admitted.

The PCOE nurses open special follow-up files containing patient's details as well as their anthropometric measurements.

Other tests done are CD4%, LFTS, U/Es and FBC as part of a pre-HAART assessment. This is usually done in confirmed HIV infected children once they are more stable (i.e. good response to treatment for conditions they came with e.g. normal temperature and able to feed) in their respective wards or in the ART clinic on outpatient review. Collection of blood for these investigations is dependent on turn-around time of the DNA PCR results.

3.3. Selection of Subjects

3.3.1. Study Population

HIV exposed children aged 18 months and younger presenting to the UTH Paediatric admission ward and meeting the criterion outlined below.

3.3.2. Inclusion Criteria

- HIV exposed children aged 0-18 months admitted to the Department of Paediatrics and Child Health.
- Informed Consent by the caregiver to enroll into the study.

3.3.3. Exclusion Criteria

- Children over 18 months
- HIV exposed infants with known DNA PCR result on admission
- Critically ill babies who needed organ support such as supplemental oxygen therapy or needed to be in the intensive care setting as determined by the admitting doctors
- Refusal to consent to enroll in the study

3.4. Sampling and Sample Size Calculation

Consecutive sampling of the first 10 HIV exposed children < 18 months admitted during the study period was selected for enrollment in the study.

The prevalence of HIV infection in the children < 18 months was estimated at 16% and at 95% Confidence level and power of 80%, the sample size calculated using EpiInfo StatCalc, version 6 is **315.** This prevalence estimate was based on the records at the UTH Department of Paediatrics for 2007.

A similar figure was obtained using the formula

$n= z^2 P(100-P)/d^2$

Were n =required sample size, z=1.96, P=an estimate of prevalence, d= desired width of confidence interval.

3.5. Procedures

New patients coming in as self referrals or referrals from peripheral clinics and hospitals were admitted at UTH as described in section 3.2. Eligible patients were identified by study assistants by perusal through patient files. Patients meeting the inclusion and exclusion criteria were enrolled into the study from Monday to Friday following an informed consent process with the caregiver. An interviewer administered structured questionnaire with an attached clinical data form which outlined specific signs and symptoms based on the signs in the WHO presumptive criteria guidelines was used for entering patients' demographic, risk factor profile and physical findings.

Children enrolled had their files checked for clinical findings of the attending Paediatricians and the children were then examined for any additional findings such as nappy rash as outlined in the structured check list by the study doctor.

Study assistants went on to draw two milliliters of whole blood from the enrolled children's peripheral veins for both CD4% and DBS card preparation. The blood was transported to the laboratory in an EDTA specimen bottle. Samples together with a unique subject identifier number were delivered to the UTH Paediatric laboratory within two hours of collection. UTH Laboratory request forms were used for DNA PCR and CD4% test requests. An aggregated manifest form upon which receipt of samples was acknowledged by laboratory staff was maintained by study assistants. The specimen was then processed as described under chapter 4.

Quality control measures employed in this study were as follows:

- UTH Paediatric and KS-HHV8 laboratory staff counter-checked information on the sample and the unique subject identifier form to ensure that prior to processing, information tallied. In the event that a discrepancy was discovered, the Principal Investigator was immediately contacted and the sample was quarantined pending clarification.
- checking for volumes of samples as well as sample integrity.
- Allocating of laboratory accession numbers to samples.

Information was collected by the study assistants who were also PCOE nurses. The consent forms and the questionnaires were stored in a lockable cabinet in the linkage room. The filled out data forms were collected by the principal investigator at the end of each week for verification and entry into access dataset. Data was then analyzed with SAS, version 9.1.3.

3.6. Data Management and Analysis

3.6.1 Measurement of variables

The variables measured in the study included independent and dependent variables.

Independent variables

- **WHO presumptive criteria**; oral thrush, severe sepsis and/or pneumonia as defined by IMCI guidelines (appendix)
- **Other clinical conditions** such as Gastroesophageal reflux disease (GERD-defined as excessive vomiting, cough and airway obstruction after feeds) [46], nappy rash, splenomegaly, hepatomegaly, and lymphadenopathy
- **Other characteristics**; Age in months, Weight, length or height, exposure to PMTCT or breastfeeding
- **Laboratory variables**; CD4%. The CD4% cut-offs used where <25% in children less than 12 months old and <20% in children older than 12 months but younger than 18 months old. These are age specific cut-offs for CD4% based on the WHO immunologic criteria for disease severity.

Dependent /Outcome variable

- HIV status confirmed by DNA PCR

3.6.2. Statistical methods

- Sensitivity, specificity, positive and negative predictive value of WHO criteria (these were measured by use of 2 by 2 tables comparing the results of the WHO presumptive criteria and the DNA PCR).
- Sensitivity, specificity, positive and negative predictive value of WHO criteria plus CD4% categories and other listed clinical signs including the age of the patient.
- Multivariate analysis to identify predictors.
- T-test to compare the median CD4% in those with and without confirmed HIV.

3.6.3. Data Analysis

Data was analyzed using with SAS, version 9.1.3. and employing the statistical methods outlined above.

3.7. Ethics

Permission for the study was sought from the University Of Zambia Research Ethics Committee (REC) and the University of Alabama Institutional Review Board.

All patients were enrolled on a voluntary basis following detailed informed consent explaining all aspects of the study. No procedures were performed on the patients apart from the ones pertaining to patients routine hospital care. Care givers were assured of continued quality care if they declined to consent or withdrew at any time during the study. Care-givers were not coerced into participating in the study and no payment was given to the study participants. Strict confidentiality was maintained at all times by use of unique identity study numbers during the study and where names were used for ease of identification of results in the lab, strict confidentiality was ensured and the subjects rights were respected at all times. Laboratory findings were made available to the patient and the treating Pediatricians and kept in the ART files for appropriate follow up.

CHAPTER 4

4.0 Laboratory

4.1 Introduction.

The UTH Paediatric laboratory and the KS-HHV-8 laboratory located within the UTH Paediatric department were chosen as testing sites for CD4% and DBS for HIV DNA PCR respectively. Consideration was given to the credibility of the testing laboratories. The considered factor was the laboratory standing at international level. The External Quality Assessment (EQA) performance was used as the yardstick.

4.2 Sample processing.

Procedure employed was as outlined in the laboratory Standard Operating Procedures (SOPS). Upon receipt of blood in the UTH Paediatric lab, the blood was separated for CD4% assessment and a preparation of a DBS sample that was then delivered to the KS-HHV8 lab.

4.2.1 CD4% estimation

CD4% was estimated using the BD FACSCalibur™ flow cytometer.

Equipment and material used were

- BD FACSCalibur™ cytometer
- BD Facs Cleanse
- BD Facs Rinse
- BD FACSFlow™
- True count control
- BD Calibrite™
- Pipettes and pipette tips for delivering 20, 50 and 450µL volume
- Vortex mixer
- BD Tritest CD3 FITC/CD4 PE/CD45 PerCP with BD TruCount Tubes
- BD FACS™ lysing solution

For each patient sample, blood was labeled in a BD TruCount tube with a sample identification number. The BD trucount bead pellet was retained within the metal retainer at the bottom of the tube. 20uL of BD Tritest CD3/CD4/CD45 reagent was pipetted into the bottom of the tube and then 50µL of well-mixed, anticoagulated whole blood was pipetted into the bottom of the tube. The tube was then capped and vortexed gently to mix. This was then incubated for 15 minutes in the dark at 20-25^{o}C. The sample was then run on the BD FACSCalibur using Multiset^{TM} software for CD4 enumeration.

4.2.2 HIV DNA PCR testing

HIV DNA PCR was performed with a DBS-based qualitative technique using the Roche AMPLICOR HIV-1 Test, version 1.5. This was in accordance with the SOP entitled: Early Infant Diagnosis of HIV, SOP No: 7, UTH KS-HHV-8 Laboratory.

The HIV DNA PCR test involved four major processes namely

- sample preparation,
- PCR amplification of target DNA using HIV-1 specific complimentary primers,
- Hybridization of amplified products to oligonucleotide probes specific to the target
- Detection of the probe-bound amplified products by calorimetry

The test permits simultaneous PCR amplification of HIV-1 target and HIV-1 internal control DNA. The detection of amplified DNA is performed using target-specific oligonucleotide probes that permit the independent identification of HIV-1 amplicon and HIV-1 internal control amplicon.

4.3 Scoring of Global Results

Results for CD4 testing were reported as CD4 absolute count of cells/µL of blood and percent (%) with a reference range of 500-1400cells/µL and 31-60% respectively.

For HIV DNA PCR results, reporting was as follows:

- HIV-1 DNA detected: If HIV-1 optical density (OD) >=0.8 (regardless of IC OD)
- HIV-1 DNA not detected: If HIV-1 OD < 0.2 and IC OD >=0.2
- Invalid: If HIV-1 OD <0.2 and IC OD <0.2, needs repeat
- Indeterminate: If HIV-1 OD is between 0.2 and 0.8, and/or if HIV-1+ sample is in a well next to a positive control, or if a new HIV-1+ sample is next to a well with a previously known HIV-1+ sample, needs Retest.

4.4 Result documentation.

Compiled results were recorded on Excel worksheets and signed out by the Laboratory Manager for each laboratory. Hard copies of compiled results were delivered by the two laboratories to the Principal Investigator.

4.5 Disposal of blood samples.

Tested blood samples for CD4% were kept for a period of 24hrs and thereafter discarded as per laboratory procedure. For HIV DNA PCR, the wastes accumulated were deposited in the waste collection containers prior to daily emptying for disposal as per laboratory procedure.

CHAPTER 5

5.0. General Description of Results

Between May and November, 2009, 6,816 children presented to the UTH of whom 3905 (57%) were infants aged less than 18 months. Nineteen per cent (19%) or 737 of these children tested positive by rapid HIV tests used according to the National HIV testing protocol.

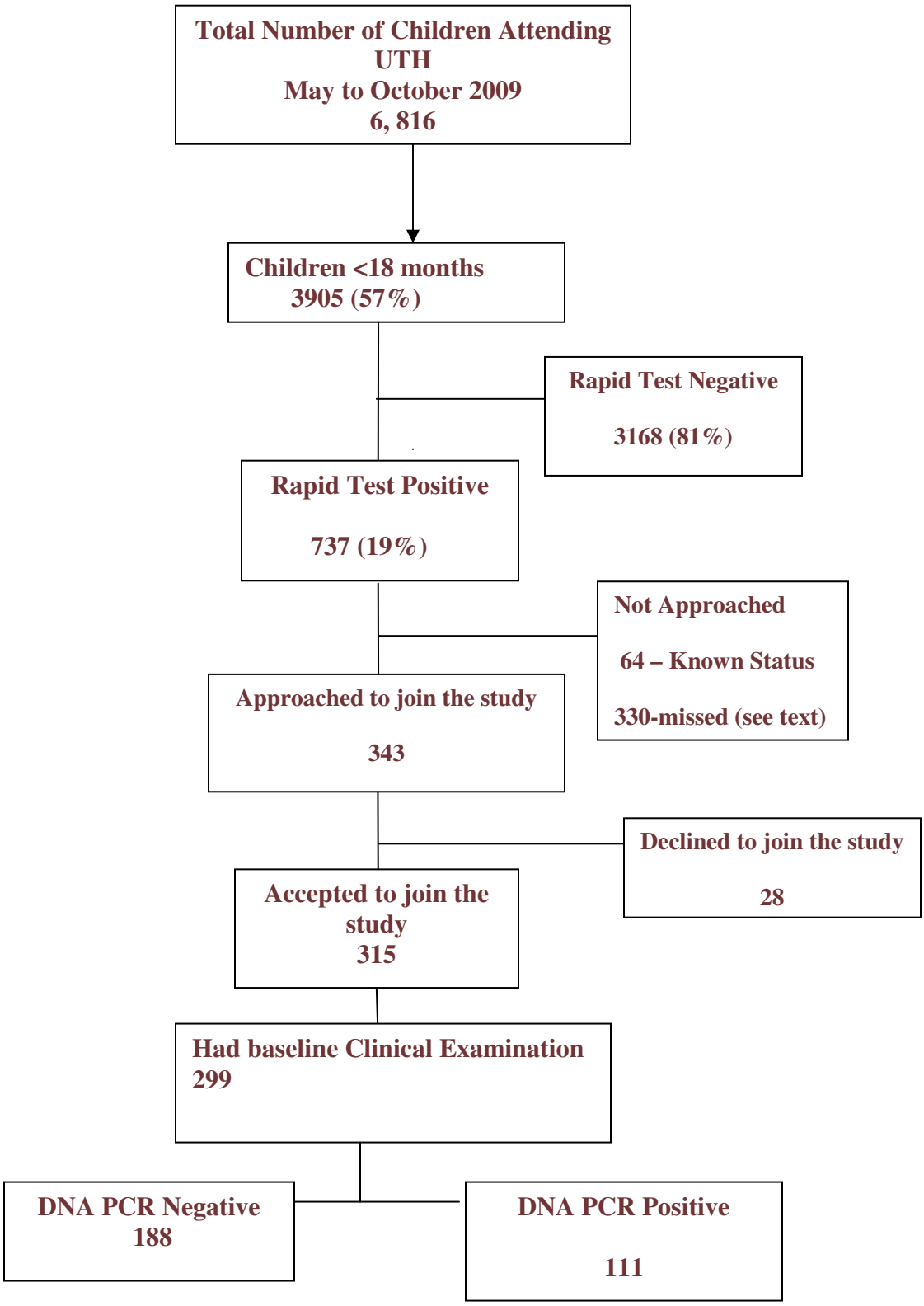
Of the 737 seropositive patients, 394 positive patients were not approached to participate in the study. Sixty four (64) of these were of known HIV DNA PCR status as they had tested in the previous admissions or at the referral centre. The rest (330) were missed because they were too ill or the guardian was not the child's biological parent and had little information about the child or were discharged before they could be approached to participate in the study.

Of the 343 patients approached to join the study, 315 (92%) agreed to participate. Ten (10) patients declined to enroll in the study because their caregivers felt that too much blood had been already been drawn prior to being approached to participate in the study while eleven (11) needed to consult their spouses. Seven (7) patients agreed to participate in the study but were not enrolled as they were discharged before being consented.

5.1. Prevalence of HIV by DNA PCR.

315 patients had consents and questionnaires filled but 16 of these were excluded from final data analysis due to incomplete CD4% or DNA PCR results. Of the 299 caregiver-infant pairs analyzed, 111 (37%) were HIV DNA PCR positive (Figure 1).

Figure 1 Flow chart for the selection of Study subjects



5.2. Baseline Characteristics of Caregivers.

Biological mothers accounted for 94% of the respondents with the majority aged between 25-35 years (57.4%). A small minority (5.4%) had had no formal education though the majority 181 (61%) were unemployed with only 18% being in formal employment. Information on the use of PMTCT was available for 282 mother-infant pairs and 139 (49%) of these had both mother and baby receiving ARVS (AZT and /or NVP or mother on HAART) for PMTCT. 122 (43%) did not receive any form of PMTCT and in 13% of these, drugs were only given to the mother. Drugs used for PMTCT were AZT and / or NVP or HAART (Table 1).

5.3. Baseline Characteristics of the Children

227 (75.9%) of the children enrolled in the study were aged between 0-6 months with the majority (53.3%) being male. Only 4.3% were aged between 12-18 months. 293 (98.0%) had a living biological mother while 22% reported death of a sibling. The commonest reason for admission was pneumonia (39.5%) followed by sepsis (31.4%). Forty nine percent (49%) of patients enrolled were on cotrimoxazole prophylaxis for Pneumocystis Jerovici. The cotrimoxazole prophylaxis was started at the primary health care facilities prior to referral of the children to UTH (Table 2).

5.4. Performance of Different Algorithms on Diagnosis of HIV

The sensitivity of the WHO-PDC criteria used on its own was 46% and when combined with CD4% results, sensitivity was found to increase to 81%. This change in sensitivity was seen for CD4% <25% in children less than 12 months old and CD4% <20% in children older than 12 months but younger than 18 months old. Combining WHO-PDC criteria with CD4% and clinical parameters increased the sensitivity to 86%, while IMCI sensitivity was found to be 14% on its own and 73% when combined with CD4% (Table 3 and Figure 2). The specificity was high in all these situations (Table 3). The WHO-PDC criteria showed increased sensitivity with increasing age (Table 4). Most of the children with a positive PCR result were in the 0-6 months age group, which also accounted for the majority of the study participants (Table 5).

5.5. Effect of PMTCT

Of the 111 HIV infected patients, 50.5% had received drugs for PMTCT. Of the 180 uninfected children, 62% received drugs for PMTCT while 38% did not. PMTCT was not a significant factor for HIV infection (Odds ratio; 0.62; 95% CI = 0.37 to 1.03).

5.6. Effects of breastfeeding

Of the 111 DNA PCR positive children, 72 (64.9%) had breastfed while 39 had not. 58.3% of the uninfected children (187) had breastfed while the rest had not. In this study, breast feeding was also not significant for HIV infection (Odds ratio; 1.32; 95%CI=0.79to2.21).

Figure 2 Receiver Operating Curves for HIV-1 Diagnostic Algorithms

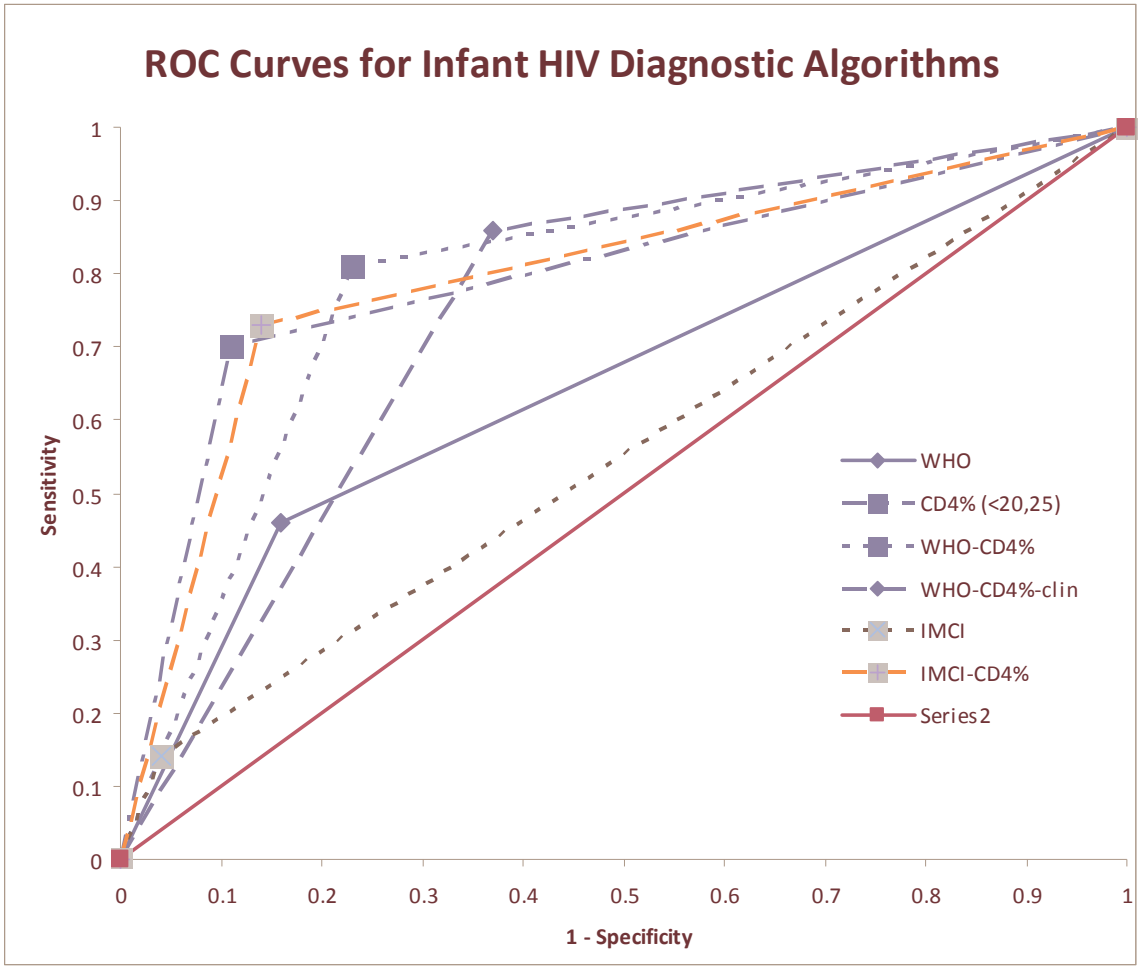


Table1. Baseline characteristics of the 299 enrolled caregiver		
Characteristic	**N**	**Percent**
Age of caregiver, years		
15-25	92	31.0%
25-35	171	57.4%
35 and above	35	11.7%
Relationship to the child		
Mother	280	94%
Father	3	1%
Aunt	6	2%
Uncle	10	3%
Education level		
Tertiary	28	9.3%
Secondary	137	45.2%
Primary	118	39.5%
No formal education	16	5.4%
Employment status		
Employed	55	18%
Unemployed	181	61%
Self employed	62	21%
ARVs for PMTCT		
Used no ARVs	122	43.3%
Only mother received	21	7.4%
Both mother and infant received	139	49.3%

Table 2. Baseline characteristic of the 299 children enrolled and tested for HIV-1		
a. Characteristic	**N**	**Value**
Age category, n (%)		
0-6 months	227	75.9%
6-12 months	59	19.7%
12-18 months	13	4.3%
CD4% for all children, median (IQR)		
all children	299	31(20-43)
children < 12 months of age	286	32(20-44)
children > 12 months of age	13	20(13-27)
CD4% for HIV infected children, median (IQR)		
all children	111	18(12-26)
Children < 12 months of age	98	18(12-26)
Children > 12 months of age	13	20(13-27)
CD4% for HIV uninfected children, median (IQR)		
all children	188	40(31-50)
children < 12 months of age	188	40(31-50)
children > 12 months of age	**0**	-
Sex, n (%)		
Male	159	53.2%
Female	140	46.8%
Status of relative		
Children whose Mothers were alive	293	98.0%
Had sibling death	67	22.5%
b. Diagnosis at admission, n (%)		
Pneumonia	117	39.5%
Excessive irritability/fever	19	6.4%
Chronic diarrhea	4	1.3%
Sepsis	93	31.4%
Oral thrush	3	1.0%
URTI	7	2.4%
Other^	53	17.9%
c. Co-trimoxazole prophylaxis	118	49%

Other ^ included children with acute diarrhoeal disease, acute severe malnutrition, meningitis and hydrocephalus.

Table 3. Sensitivity, Specificity and predictive values for HIV-1 infection in infants using Different Algorithms				
Criteria	Sensitivity	Specificity	PPV	NPV
WHO-PDC	0.46 (0.37 to 0.55)	0.84 (0.78 to 0.89)	0.62 (0.52-0.73)	0.72 (0.66-0.78)
CD4% alone	0.70 (0.62 to 0.79)	0.89 (0.85 to 0.94)	0.80 (0.72-0.88)	0.84 (0.78-0.89)
WHO-PDC and CD4%	0.81 (0.74 to 0.88)	0.77 (0.71 to 0.83)	0.67 (0.59-0.75)	0.87 (0.82-0.92)
WHO-PDC and Hepatomegaly	0.47 (0.38 to 0.56)	0.81 (0.75 to 0.86)	0.59 (0.49-0.69)	0.72 (0.66-0.78)
WHO-PDC and Splenomegaly	0.47 (0.38 to 0.56)	0.84 (0.78 to 0.89)	0.63 (0.52-0.73)	0.73 (0.67-0.79)
WHO-PDC and Nappy rash	0.47 (0.38 to 0.56)	0.84 (0.78 to 0.89)	0.63 (0.52-0.73)	0.73 (0.67-0.79)
WHO-PDC and Hepatomegaly and Splenomegaly	0.48 (0.38 to 0.57)	0.81 (0.75 to 0.86)	0.60 (0.49-0.70)	0.72 (0.66-0.78)
WHO-PDC and Lymphadenopathy	0.50 (0.40 to 0.59)	0.81 (0.76 to 0.87)	0.61 (0.51-0.71)	0.73 (0.67-0.79)
WHO-PDC and Hepatomegaly and Splenomegaly and lymphadenopathy	0.51 (0.42 to 0.61)	0.79 (0.73 to 0.85)	0.59 (0.49-0.69)	0.73 (0.67-0.79)
WHO-PDC and Weight < 3rd percentile	0.56 (0.47 to 0.65)	0.73 (0.67 to 0.80)	0.55 (0.46-0.65)	0.74 (0.67-0.80)
WHO-PDC and weight < 3rdpercentile and hepatomegaly and splenomegaly and lymphadenopathy and CD4%	0.86 (0.79-0.92)	0.63 (0.56-0.70)	0.58 (0.50-0.65)	0.88 (0.83-0.94)
IMCI	0.14 (0.10-0.20)	0.96 (0.94-0.99)	0.68 (0.49-0.88)	0.65 (0.60-0.71)
IMCI + CD4%	0.73 (0.65-0.81)	0.86 (0.81-0.91)	0.76 (0.68-0.84)	0.84 (0.79-0.90)

Table 4. Performance of WHO-PDC of HIV-1 infections in infants by age		
Age Group in months	**Sensitivity (95% CI)**	**Specificity (95% CI)**
0 – 6	0.37 (0.24 to 0.49)	0.84 (0.78 to 0.89)
6 -12	0.49 (0.33 to 0.64)	0.83 (0.66 to 1.0)
12 – 18	0.77 (0.54 to 1.0)	-

Table 5. The positive PCR results by age group	
Age in months	**Frequency (per cent)**
0- 6	57 (51)
6- 12	41 (37)
12-18	13 (12)

CHAPTER 6

6.0. Discussion

Against a background of high infection rates and unavailable equipment for making a definitive diagnosis of HIV infection in infants and young children, clinical algorithms have been employed. This use of clinical algorithms is for the purpose of commencing ART in the absence of HIV DNA PCR results. In this study, the performance of the WHO PDC was assessed in children less than 18 months in making the diagnosis of HIV. This was correlated with the DNA PCR which is the current gold standard for the diagnosis of HIV in children less than 18 months. The HIV prevalence was 37% (111) in this group of 299 seropositive children. The WHO-PDC was found to be 46% sensitive with a specificity of 84%. Sensitivity of the criteria improved slightly with increase in age above six (6) months of age. A low CD4% on its own or combined with the WHO-PDC was found to be highly predictive of HIV infection. Clinical signs proved to be 47% to 56% sensitive when combined with WHO-PDC despite being highly specific when no more than three signs were present. Use of the IMCI guidelines also showed an increased sensitivity (73% from 14%) when combined with the CD4%.

6.1. Prevalence of HIV infection

We found an HIV prevalence of 37% by DNA PCR in this study group. In the Kenyan study, prevalence was 60%.[45] No clear explanation could be found for this difference in prevalence between the two studies as the children had similar age characteristics and both studies were done during the PMTCT era.

6.2. Performance of Different Algorithms on Diagnosis of HIV

We found the WHO-PDC sensitivity of 46% and a specificity of 84%. This would identify 51 of the 111 patients who are infected with HIV. This compares favorably with the Kenyan study in which the sensitivity was 43% and specificity 88% implying that the WHO-PDC is highly insensitive in low resource environments and as such, other parameters have to be considered to increase sensitivity. [45]

The specificity is however high in both cases showing that the algorithm would correctly identify as uninfected those children who are HIV negative.

The CD4% was stratified according to WHO immunological criteria for severe immunodeficiency (<25% in those <12 months and <20% in those <18 months but >12 months).[4, 5] In this study, CD4% was found to strongly predict the chances of being HIV positive whether used alone (70% sensitivity at 95% CI 0.62 to 0.79) or in combination with the WHO-PDC (81% sensitivity at 95% CI 0.74 to 0.88). Analyzing WHO-PDC and CD4% criteria would have identified 88 (79%) of the 111 children infected with HIV. We found that a low CD4% was likely to give a positive DNA PCR result. Again, the sensitivity of 81% is similar to the findings in the Kenyan study in which 84% were correctly identified as infected. The specificity was however much higher in our study at 89% for CD4% alone, and 77% when CD4% was combined with WHO-PDC unlike the 39% and 41% respectively, noted in the Kenyan study. This is regardless of the fact that blood for CD4% was collected during an acute illness in both studies. We however, could not establish if the prevailing infections had a marked reductive effect on the T-lymphocytes. It would have also been useful to compare the CD4% to the CD8% as the CD4:CD8 ratio has been noted to be a more sensitive predictor of HIV infection in exposed children in other areas.[46-49]

The median CD4% for the entire study cohort of exposed children was 31%. The median CD4% in HIV infected children was 18% whereas it was 40% in the HIV negative children. This implies that a seropositive child with a low CD4% can benefit from early initiation of ART as DNA PCR results are awaited. Various studies have also shown that CD4% is low in HIV-1 infected children and is highly predictive of disease progression even in young infants.[50-52] An earlier study done in Zambia showed a median CD4% of 35% in HIV negative children less than 2 years whilst our study had a CD4% of 40% in the HIV DNA PCR negative cohort.[53]

Another study done on Zambian children who were malnourished showed that CD4 values continued to decline in HIV infected children despite nutritional recovery as compared to those who were not infected. [54]

The trend in the performance of the WHO-PDC in infants by age showed an increase in sensitivity from 37% to 49% for ages 0-6 months and 6-12 months respectively. The results were not significant in the 12-18 months range probably because of the small number of patients in this age group. The increase in sensitivity with increase in age may be explained by the fact that those who are perinatally infected may be showing signs and symptoms of HIV infection which can be picked on examination while the small number in the 12-18 months age-group may show that some of these patients may have died by then in the absence of HAART. This was confirmed by a pooled analysis on mortality of HIV infected children born to HIV-1 infected mothers in Africa which showed 30% mortality by the age of 1 year and 50% by the age of 2 years without HAART.[11] Equally, the increase in sensitivity could also be due to the high possibility of a positive ELISA test being reflective of the true immunological status of the child as opposed to maternal antibodies that would be present in younger children. There is also a possibility that the older children would have already been picked up by DNA PCR testing as this service is already being provided in Lusaka.

Use of DNA PCR in the diagnosis of HIV yielded 51% positive results in the 0-6 month's age group whereas the WHO-PDC in this age group only picked 37 % of those infected and missed 14%. This could be due to paucity of positive physical signs in these children. The WHO-PDC is centered on the presence of physical signs[3]. Infants aged less than six months rarely present with AIDS defining illness to enable a clinician make a presumptive diagnosis of HIV.

Additionally, in this study we found that presence of nappy rash or splenomegaly or hepatomegaly did not improve the sensitivity of the WHO-PDC algorithm (47% for all at 95% CI 0.38 to 0.56). This is unlike the South African study in which nappy rash was significantly ($p<0.05$) more common in HIV infected children. [55] It can be presumed that

nappy rash is common to both HIV infected and uninfected children in our set up as most caregivers may not be in a position to provide nappies to the children due to the high unemployment levels (Table 1), though this association would require more detailed analysis. Weight < 3rd percentile was not a sensitive indicator of HIV infection (46%) despite being specific (84%) with a PPV and NPV of 62% and 72% respectively. The South African study showed that weight in the bottom 10% of their cohort was significant as predictor of HIV ($p<0.001$).[55] It should be noted, however, that HIV influenced the nutritional status of these children and this correlates with the findings of a Zambian study done in 2001 in which weight for height Z-score post oedema was found to be significantly lower in HIV seropositive patients when compared to those who were seronegative.[56]

When the WHO-PDC, weight<3rd percentile, hepatomegaly, splenomegaly, lymphadenopathy and CD4% were combined, the sensitivity improved to 86%, though specificity reduced to 63% with a PPV 58% and NPV of 88%. This shows that the clinical algorithm is only a good predictor of HIV infection if a combination of signs is present. This implies that patients would be identified when the HIV infection has advanced and would be of limited help.[9]

Analysis using the IMCI guidelines yielded 14% sensitivity when compared to the DNA PCR. When combined with CD4%, sensitivity improved to 73% with a specificity of 86%. This would have identified 81 of the 111 HIV infected patients again showing that the use of CD4% would increase the number of HIV infected patients identified using the IMCI tool. Thus, in the presence of CD4% the IMCI tool would be useful in situations where caregivers are more familiar with the IMCI guidelines as opposed to the WHO-PDC.

6.3. Effect of PMTCT

In this study, PMTCT was not a significant factor for HIV infection (Odds ratio; 0.62; 95% CI = 0.37 to 1.03). This does not compare with other studies that have shown that use of ARVs for PMTCT reduces HIV transmission in HIV exposed children. [22, 26, 27] These findings could be because the study was not powered to accurately measure the risk of getting infected if one did not go through PMTCT nor did it specifically analyze for the regimen that would be most effective in preventing transmission of HIV.

6.4. Effects of breastfeeding

In our study, breast feeding was not a significant risk factor for HIV infection (Odds ratio; 1.32; 95% CI=0.79 to 2.21). This could be explained by the fact that data was not verified and the respondents may have told the study assistants what they thought they wanted to hear. Our study did not analyze the risk based on the duration of the breastfeeding and the levels of mixed feeding were also not assessed. An earlier study done in Zambia to examine the safety of breastfeeding in HIV exposed infants estimated that 84% of the infected women admitted to have exclusively breastfed for the first four months and this was said to be protective against HIV infection.[57] Mixed feeding increased the likelihood of being HIV infected.[58, 59]

CHAPTER 7

7.0. Conclusion

Adding CD4% testing to the WHO PDC clinical algorithm improved the sensitivity of the algorithm from 46% to 81%. The WHO-PDC was more sensitive in children over 6 months of age. However, DNA PCR still remains the most reliable tool in detecting HIV infection especially in the 0-6months age group. Further studies to validate the information obtained from this study are required in non tertiary institutions where transportation and loss of results pose major problems.

7.1. Limitation of the Study

The study site was a tertiary hospital which deals with patients with complications. The study population was thus not representative of the general population seen at primary care settings. This could thus explain why most of the children with positive PCR were in the 0-6 months age group as this was the most represented age group (75.9%).

7.2. Benefits of the Study

The study has provided information about the use of CD4% that may strengthen the criteria for presumptive diagnosis of HIV in children <18 months for the purpose of early initiation of ART in areas where DNA PCR is not available. Of benefit to the study subjects was the reduced turnaround time for DNA PCR results from the usual two to three weeks to one week in this study allowing for earlier decision making by the patient's clinician.

7.3. Recommendations

The use of CD4% to improve the sensitivity of the WHO clinical algorithm as shown in this study should be assessed in a primary care setting where selection bias is removed.

15.0 REFERENCES

1. HIV paediatric Prognostic Markers Collaborative Study Group. Short-term risk of disease progression in HIV-1 infected children receiving no antiretroviral therapy or Zidovudine monotherapy: a meta-analysis. *Lancet* 2003 Nov 15; **362**(9396): 1605-11.

2. HIV paediatric Prognostic Markers Collaborative Study Group. Current CD4 cell count and short term risk of AIDS and death before availability of effective antiretroviral therapy in adults and children; a meta-analysis. *J Infect Dis* 2008 Feb 1; **197**(3): 398-404.

3. ART of HIV infection in infants and children in low resource setting. WHO 2006

4. Violari A, Cotton M, Gibb D, Babiker A, Steyn J et al. Children with HIV Early Antiretroviral Therapy (CHER study). *N Engl J Med* 2007; **359**(21): 2233-44

5. Faye A, Le Chenadec J, Dollfus C, Thuret, et al. Early versus Differed Antiretroviral multidrug therapy in HIV type 1. *Clin Infect Dis* 2004; **39**: 1692-98.

6. Paediatric European Network for Treatment of AIDS (PENTA). Highly active antiretroviral therapy started in infants under 3 months of age. *AIDS* 2004; **18**: 237-245.

7. World Health Organization, United Nations Children's fund. Integrated management of childhood illnesses complimentary course on HIV/AIDS Geneva; WHO 2006 Jan.

8. Horwood C, Liebeschuetz S, Blaauw D, Cassol S, Qazi S. Diagnosis of paediatric HIV infection in a primary Health Care Setting with a clinical Algorithm. *Bull World Health Organ* 2003; **81**(12): 858-66.

9. Jones SA, Shermann GG, Coovadia AH. Can Clinical Algorithms Deliver an Accurate diagnosis of HIV Infection in infancy? *Bull World Health Organ* 2005 Jul; **83**(7): 559-60.

10. Dabis F, Elenga N, Meda N, Leroy V, Viho I, Manigart O, et al ;18 Month mortality and perinatal exposure to Zidovudine in West Africa. *AIDS* 2001 April, 13; **15**(6): 771-9.

11. Newell ML, Coovadia H, Cortina-Borja M, Rollino N, Gaillard P, Dabis F. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. *Lancet* 2004 Oct 2-8; **364**(9441): 1236-43.

12. MOH –personal communication.

13. *2006 Report on the global AIDS epidemic.* Geneva, UNAIDS, 2006 (http://www.unaids.org/en/HIV_data/2006GlobalReport/default.asp).

14. UNICEF Stock taking report on children and AIDS; unit against children, unit against AIDS, January, 2007.

15. United Nations Children's Fund. Children and AIDS. Third Stocktaking Report, 2008. New York, NY; UNICEF, 2008. (http;//www.unicef.org/publications).

16. Central Statistics Office (CSO) Zambia, HIV/AIDS epidemiological projections. 2004.

17. UTH Pediatrics Department Statistics, 2007.

18. Chintu C, Luo C, Bhat G, Du Pont HL, Mwansa Salamu P, Kabika M et al; Impact of the human immunodeficiency virus type-1 on common paediatric Illnesses in Zambia. *J. Trop. Pediatr* 1995; **41**(6):348-53.

19. De Cock KM et al. Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. *JAMA* 2000, **283**(9):1175-82.

20. Sinkala M, McFarlane Y, Nguni C, Stringer J,Mphanza E, Myzece E, Stringer E. Scale up of PMTCT services in Zambia. 2005, Lusaka DHMT, CIDRZ and UAB.

21. Read J et al. A prospective cohort study of HIV-1-infected pregnant women and their infants in Latin America and the Caribbean: the NICHD International Site Development Initiative Perinatal Study. 12th Conference on Retroviruses and Opportunistic Infections. Boston, MA, USA. 22–25 February 2005 (Abstract 790).

22. Mother-to-child transmission of HIV infection in the era of highly active antiretroviral therapy. *Clin Infect Dis* 2005; **40**(3): 458-65.

23. Dorenbaum A, Cunningham CK, Gelber RD, Culnane M, Mofenson L, Britto P, Rekacewicz C, Newell ML,Delfraissy JF, Cunningham-Schrader B, Mirochnick M, Sullivan JL. Two-dose intrapartum/newborn niverapine and standard antiretroviraltherapy to reduce perinatal HIV transmission: a randomized trial. *JAMA* 2002; **288**(2): 189-98.

24. Stanton CK, Holtz SA. Levels and trends in cesarean birth in the developing world. *Stud Fam Plann* 2006; **37**(1):41–48.

25. Mbewe M, Bolton C, Levy J, Sinkala M, Bulterys M, Chi BH et al; Children Enrolled in a Public HIV care and treatment programme in Lusaka, Zambia;

Rapid Scale up and 1st year clinical outcomes. *AIDS 2006* XVI International AIDS conference. Abstract no. MOABO201.

26. Shapiro R et al. Maternal single-dose niverapine vs. placebo as part of an antiretroviral strategy to prevent mother-to-child HIV transmission in Botswana. *AIDS* (in press).

27. Lallemant M et al. Single-dose perinatal niverapine plus standard Zidovudine to prevent Mother-to-child transmission of HIV-1 in Thailand. *N Engl J Med* 2004, **351**(3) :217-28.

28. Gaillard P et al. Use of antiretroviral drugs to prevent HIV-1 transmission through breast-feeding: from animal studies to randomized clinical trials. *JAIDS* 2004; **35**(2):178-87.

29. Behram E.R, Kliegman R.M and Jenson B.H; Nelson Text Book of Pediatrics 2004; **17**: 1113-18.

30. Center for Disease Control; Revision of the CDC Surveillance Case definition for acquired immunodeficiency syndrome. Council of state and Territorial Epidemiologists, AIDS program, Center for Infectious Disease. *MMWR Morb Mortal Rep* 1987; **36**(1):1s-15s.

31. Centres for Disease Control. Classification systems for Human immunodeficiency virus (HIV) infection in children under 13 years of age. MMWR Morb Mortal Wkly Rep.1987; **36**: 225-30,235-36.

32. Centres for Disease Control 1993 revised classification system for HIV infection and expanded Surveillence Case definition for AIDS among adolescents and adults. *MMWR Recomm Rep* 1992; **41**(RR-17):1-19.

33. World Health Organization, WHO case definition of HIV for surveillance and Revised Clinical Staging and Immunological Classification of HIV-related disease in adults and children. Geneva, Switzerland: WHO; 2006. (www.who.int/hiv/pub/guidelines/hivstaging/en).

34. World Health Organization , Scaling up Antiretroviral Therapy in Resource-Limited Settings; Treatment guidelines for a Public Health Approach.2003, Revision, Geneva, Switzerland; WHO 2004. (www.who.int/hiv/pub/prev.care/en/arvrevison).

35. World Health Organization, Division of Diarrhoeal and Acute Respiratory Disease Control, Integrated Management of the sick child. *Bull World Health Organ* 1995; **73**: 735-40.

36. Lepage P, Van der Perre P, Dabis F,et al. Evaluation and Simplification of the WHO clinical case definition for paediatric *AIDS* 1989 Apr; **3**: 221-25.

37. Centres for Disease Control and Prevention.1994 revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR. Recomm Rep* 1994;**43**(RR-12): 1-10.

38. Shilalukey-Ngoma M.P, Mushanga A, Wang X, Watanabe M- Clinical presentation of HIV/AIDS in high risk Neonate in Zambia. *Early Hum Dev* 1992; **3**: 221-4.

39. Obimbo E, Mbori-Ngacha D, Ochieng JO, Richardson BA, Otieno PA, Bosire R, Farquhar C, Overbaugh J, John-Stewart GC. Predictors of Early Mortality in a Cohort of Human immunodeficiency Virus type 1- infected African children. *Pediatr Infect Dis J* 2004; **23**(6): 536-43.

40. Walker S.A, Mulenga V, Sinyinza F, Lishimpi K, Nunn A, Chintu C, Gibb DM and the CHAP Trial Team, Determinants of Survival Without Antiretroviral Therapy After Infancy in HIV -1 Infected Zambian Children in the CHAP trial; *JAIDS* 2006; **42**;637-45.

41. World Health Organization. Acquired immunodeficiency syndrome (AIDS) WHO/CDC case definition for surveillance. *Wkly Epidemiol Rec* 1986; **61**: 69-73.

42. World Health Organisation.AIDS:1987 revision of CDC/WHO case definition. *Bull World Health Organ* 1988; **66**:259-63, 269-73.

43. World Health Organization. Interim proposal for a WHO staging system for HIV infection and disease. *Wkly Epidemiol Rec* 1990; **65**: 221-24.

44. World Health Organization case definitions for AIDS surveillance in adults and adolescents. *Wkly Epidemiol Rec* 1994; **69**: 273-75.

45. Inwani I, Mbori-Ngacha D, Nduati R, Obimbo E, et al; Performance of Clinical Algorithms for HIV-1 Diagnosis and Antiretroviral Initiation Among HIV-1 Exposed Children Aged less than 18 months in Kenya. *JAIDS* 2009; **50**: 492-98.

46. Shearer WT, Patiwa S, Read J.S, Chen J, Wijayawardana SR, Palumbo p,et al, CD4/CD8 T-cell ratio predicts HIV infection in infants; The National Heart, Lung and Blood Institute P2C2 study. *J. of Allergy and Clinical Immunology* 2007 Dec; **120**(6): 1149-56.

47. Moodley D, Bobat RA, Coovadia HM, Doovasamy T, Munsamy S and Gouws E, Lymphocyte subset changes between 3 and 15 months of age in infants born to HIV seropositive women in South Africa. *Trop Med Int Health* 1997 May; **2**(5): 415-21.

48. Zijenah LS, Katzenstein DA, Nathoo KJ, et al.T lymphocytes among HIV-1 infected and uninfected infants:CD4/CD8 ratio as a potential tool in diagnosis of infection in children under 2 years. *J Transl Med* 2005;**3**; 3-6.

49. Embree J , Bwayo J, Nagelkerke N, et al. Lymphocyte subsets in human immunodeficiency virus type 1 infected and uninfected children in Nairobi. *Pediatr Infect Dis J* 2001; **20**:397-403.

50. Mofenson LM, Korelitz J, Meyer WA 3rd, Bethel J, Rich K, Pahwa S, Moye J Jr, Nugent R,Read J. The relationship between serum human immunodeficiency virus type-1 (HIV-1) RNA level, CD4 lymphocyte percent, and long-term mortality risk in HIV-1 infected children. National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial group. *J Infect Dis* 1997; **175**: 1029-38.

51. Koracs A, Frederick T, Church J, et al. CD4 T-lymphocyte counts and Pneumocystis Carinii Pneumonia in Pediatric HIV infection. *JAMA* 1991; **265**: 1698-1708.

52. Dunn D. Short term risk of disease progression in HIV-1 infected Children receiving no antiretroviral therapy or Zidovudine monotherapy; a meta-analysis. *Lancet* 2003; **362**: 1605-11.

53. Ndhlovu Z, Ryon J, Griffin E, at al. CD4+ and CD8+ T- lymphocyte Subsets in Zambian Children. *J Trop Pediatr* 2004; **50**(2): 94-97.

54. Hughes SM, Amadi B, Mwiya M, Nkamba H, Mulundu G, Tomkins A, Goldblatt D. CD4 counts Decline Despite Nutritional Recovery in HIV-Infected Zambian Children With Severe Malnutrition. *Pediatrics* 2009 Jan; **123**(2): e347-51.

55. Jaspan H et al; Clinical and immunological characteristics of very young infants with HIV infection; Children with HIV Early Antiretroviral Study.15th conference on Retroviruses and opportunistic infections, Boston, Abstract 76, 2008.

56. Amadi B, Kelly P, Mwiya M, Mulwazi E, Sianongo S, Changwe F, Thomson M, Hachungula J, Watuka A, Walker-Smith J, Chintu C. Intestinal and Systemic Infection, HIV, and Mortality in Zambian Children with Persistent Diarrhea and Malnutrition, *J Pediatr Gastroenterol Nutr* 2001 May: **32**: 550-54.

57. Kuhn L, Sinkala M, Kankasa C, Semrau K, Kasonde P, et al. High uptake of exclusive breastfeeding and reduced early post-natal HIV transmission. PLoS ONE 2007; e1363 doi.

58. Iliff P.J, Piwoz E.G, Tavengwa N.V, Zunguza C.D. Marinda E.T, et al, Early Exclusive Breastfeeding reduces the risk of postnatal HIV Transmission and Increases HIV Free Survival. *AIDS* 2005; **19**: 699-708.

59. Coovadia HM, Rollins NC, Bland RM, Little K, Couttsoudis A, et al, Mother-to-child- transmission of HIV-1 infection during breastfeeding in the first 6 months of life; An interventional cohort study. *Lancet* 2007; **369**: 1107-16.

APPENDIX

APPENDIX 1.

Questionnaire

Title; To evaluate the sensitivity of the WHO presumptive diagnostic criteria in the diagnosis of HIV in children <18 months admitted to UTH

Investigator; Dr Kunda Kapembwa

Demographic/personal data

Identification/file number:…….

1. Age (in months):
   - a) 0-6 months ☐
   - b) 6-12 months ☐
   - c) 12-18 months ☐

2. Sex
   - a) M ☐
   - b)Female ☐

3. Relationship of respondent to the child
   - a)Mother ☐
   - b)Father ☐
   - c)Aunt ☐
   - d)Uncle ☐
   - e)Other ☐

4. Age of caregiver

a) 15-25 ☐

b) 25-35 ☐

c) 35 and above ☐

5. Higher educational status

a) Tertiary level (College/University) ☐

b)Secondary level ☐

c) Primary level ☐

d)No formal education ☐

6. Employment status of caregiver

a) Employed ☐

b) Unemployed ☐

c) Self employed ☐

Residential address.....

Cell phone no.: .....

Family History

7. Mother

a) Dead ☐

b) Alive and well ☐

c) Alive and ill ☐

8. a) If dead; cause of death

1. Prolonged illness (TB etc) ☐

2. Short illness ☐

8. b) Other (specify) …….

9. Father

a) Dead ☐

b) Alive and well ☐

c) Alive and ill ☐

10. Sibling death

a) Yes ☐

b) None ☐

c) Don't know ☐

11. a) If yes, cause of death

1. Long illness ☐

2. Short illness ☐

11. b) Other (specify)……

Information on PMTCT (only in mothers whose status is known)

12. a) Did mother go through PMTCT ……yes ☐ no ☐

12. b) If yes, what was given to the mother?

1. NVP + AZT ☐

2. NVP ☐

3. Don't know ☐

13. Is mother on HAART?

1. Yes ☐

2. No ☐

3. Don't know ☐

14. a) Was baby given drugs post delivery?

Yes ☐ No ☐ Don't know ☐

14. b) If yes, what drugs

1. NVP + AZT ☐
2. NVP ☐

Medical History

15. Number of visits to local clinic/hospital since birth

a) None ☐
b) Once ☐
c) More than once ☐

16. Number of hospital admissions

a) None ☐
b) Once ☐
c) More than once ☐

17.a) Is the child currently on any drugs?

Yes ☐ No ☐

17. b) If yes, which drugs

1. cotrimoxazole prophylaxis ☐
2. other antibiotic ☐
3. prophylaxis for PMTCT ☐
4. other (specify)……

18. Reason for current admission (diagnosis at admission)

a) Pneumonia ☐ Recurrent yes ☐ no ☐
b) Excessive irritability/fever ☐ Recurrent yes ☐ no ☐

c) Chronic Diarrheal ☐ Recurrent yes ☐ no ☐

d) sepsis ☐ Recurrent yes ☐ no ☐

e) oral thrush ☐ Recurrent yes ☐ no ☐

f) URTI ☐ Recurrent yes ☐ no ☐

g) other (specify)……..

19. Duration of symptoms

a) <1 week ☐

b) > 1 week but < 2 weeks ☐

c) Over 2 weeks ☐

20. Reason for previous hospitalisation

Pneumonia ☐ Diarrhoea ☐ Measles ☐ Malnutrition ☐

Malaria ☐ Fever ☐ Pulmonary TB ☐ URTI ☐

Failure to thrive ☐ Other(specify)……

Growth and Development

21. Birth weight.....

22. Growth pattern on under five card

a) Below the 3rd percentile ☐

b) In between 3rd and 90th percentile ☐

c) Above the 90th percentile ☐

d) Under five card not available ☐

23. Milestones (refer to Chart)

a) 0 – 4 months appropriate ☐ Inappropriate ☐ (delayed/regressed)

b) 4 – 6 months appropriate ☐ Inappropriate ☐ (delayed/regressed)

c) 6 – 9 months appropriate ☐ Inappropriate ☐ (delayed/ regressed)

d) 9-12 months appropriate ☐ Inappropriate ☐ (delayed/ regressed)

e) 12-18months appropriate ☐ Inappropriate ☐ (delayed/ regressed)

24. Fully immunised

yes ☐ no ☐ other (specify)……..

25.a) Breastfeeding yes ☐ no ☐

25. b) If No, when did the child stop breastfeeding …… months

Examination findings

General appearance

i. Wasting yes ☐ no ☐

ii. Lethargic yes ☐ no ☐

iii. Other (specify)…….

General examination

Temperature……

Weight ……

Height…….

Head circumference……

Pallor yes ☐ no ☐

Lymphadenopathy yes ☐ no ☐

Other……

Examination from head to toe;

a) Findings on the skin-(specify)……

b) Mouth and throat -oral thrush yes ☐ no ☐

-Chronic Otitis media yes ☐ no ☐

-Other (specify)……..

c) Chest -pneumonia yes ☐ no ☐

-Other (specify)…….

d) Abdomen

-hepatomegaly yes ☐ no ☐

-Splenomegaly yes ☐ no ☐

-Other (specify)……..

e) Buttocks

- wasting (baggy pants) yes ☐ no ☐

-Extensive nappy rash yes ☐ no ☐

-Other (specify)…….

f) Anal and pubic region- (specify)……..

g) Neurological Examination

- Signs of meningitis yes ☐ no ☐

- Other (specify)…….

Results

DNA PCR result negative ☐ positive ☐

Full Blood Count

CD4%

APPENDIX 2.

PATIENT INFORMATION SHEET

Title: Evaluation of the sensitivity of the WHO Presumptive diagnostic Criteria in Diagnosis of HIV in children <18 months old admitted to the paediatric department at UTH

Investigator; Dr Kunda Mutesu- Kapembwa

Introduction; I am a doctor for children working in the department of paediatrics. I am doing this research for my masters program. Kindly note that your participation in this study is voluntary.

Purpose of the study; you are being asked to have your child/ward take part in this research study. The purpose of the study is to find out if the WHO standard (WHO is an international organization which looks at the health of people in poor countries like Zambia) for finding out if a child is HIV positive is good enough to allow early treatment of HIV infection in children who are less than 18 months, and live in places where there are no medical equipment. Children who are born from a mother who is HIV positive can usually test positive because they might be carrying HIV antibodies from their mother. This is not a true reflection of their status and will therefore require to have their status confirmed by use of special medical machines(DNA PCR).This WHO standard(presumptive diagnosis) only uses the illnesses that children come to the hospital with to make a diagnosis of HIV and start ARVS (Antiretroviral Drugs).

Study procedure; This study will include only children who are less than 18 months of age, HIV antibody positive and awaiting DNA PCR result , whose caregivers consent to participate. Questions will be asked and the Childs file will be used to get the findings of the doctors. The child will then be examined for other additional

findings. 2 millilitres of blood (a teaspoon) will then collected for CD4% and FBC. This blood will be drawn from a vein in the groin (femoral Vein) or peripheries of the hands and feet (sites of cannulation). The blood will only be collected once, unless mistakes are made during the collection or the blood clots.

Possible risks and discomfort; The procedure will be short and no potential risk to the child. Your child will only experience some pain and sometimes swelling during pricks to collect blood. Bleeding may occur and necessary measures will be done to stop this as drawing blood is part of the routine hospital care in both HIV exposed and unexposed infants. Disposable needles will be used to minimise infection.

Benefits; Apart from knowing the true status, treatment for his/her HIV infection will be started the recommendations of this study will help other children in places with no DNA PCR machines to be started on treatment earlier.

Confidentiality; the information that will be collected from the research will be kept confidential. Information about the participants will be stored in a file that will not have the participants name, but a number will be assigned to it instead, The name associated with the number will be kept confidential and will not be divulged to anyone except the patients clinicians, ethics committee and the department authority if need be.

CONSENT FORM

Your participation in this study is strictly voluntary. If you refuse to take part in the study or withdraw at any time during the study, your child will still be treated without any prejudice.

Person to contact for problems or questions

If you have any problems or questions about the study, there are people you can contact. The counsellors and their staff can help you contact the right person to answer any questions you have.

For study questions, contact;

Dr Kunda Mutesu- Kapembwa
Department of Paediatrics and Child Health
University Teaching Hospital
Private Bag RW1X, Lusaka, Zambia
Mobile; 0955 758922

For questions about your rights as a research subject, contact;

The Secretary
Research Ethics Committee
Ridgeway Campus
P.O. BOX 50110
Lusaka, Zambia
Tel: 260-211-256-067

CERTIFICATE OF CONSENT

I have been invited to have my child/ ward participate in the study to evaluate the WHO criteria for making a diagnosis of HIV in children less than 18 months. Information about the study has been presented to me and I have had the opportunity to ask questions which have been answered to my satisfaction. I understand that I have the right to withdraw from the study at anytime with no penalty.

I have received a signed copy of this agreement

Name of caregiver	Date and signature
……	..……
If illiterate (thumb print)	
Name of witness	Date and signature
…….....	…….
Name of Researcher	Date and signature
……	..…….....

APPENDIX 3.

NYANJA CONSENT FORM

Pepala la chibvumekezo

Mau Ya Pamwamba

Kufufuza kukulu kusebenzesa njira ya boyanganira pau moyo watu pano padziko la pansi (WHO) kusebenzesa njira yo ganizira kupima ana onse obandwa kuli muzimai onse ali ndikalombo ka HIV ku mwana ali namyesi ikalibe kufika khumi isano ndi itatu (<18) wamene alimu chipatala chabana cha UTH.

UAB IRB Protocol Number: X090306005

Ofufuza: Dr Kunda Mutesu- Kapembwa

Opatsa ndalama: NIH/Fogarty

Chiyambi

Ndine Dotoro wa bana wamene agwilila nchito ku chipatala chabana. Ukukufufuza kuzanithandizako pa maphunziro yanga. Chonde dziwani kuti kutengako mbali kwanu mu maphunziro aya ndikozipereka.

Cholinga chamaphunziro

Nipempako kuti mwana wanu atengeko mbali muli aya maphunziro. Cholinga cha aya maphunziro nichofuna kuziba ngati chipani(WHO) chamene chilanganila pau moyo wa Bantu pano paziko lapansi mu malo yo pelewera monga Zambia, kufuna kuziba kuti mwana ngati ali nakalombo ka HIV, chingawame ku yamba musanga kumwa mankwara, kuli baja bana bali na myezi ikalibekufika pa khumi, isanu ndi itatu (<18), bamene bankala mumadela yamene yalibe zo pimira. Bana bamene ba badwa kwa a zimai ali nakalombo ka HIV, kambili aba bana anga pezeke monga ali nakalombo ka HIV chifukwa champhabvu zo tenga kwa azimai ao. Ichi sichilangiza kuti mwana ali nakalombo ka HIV, chimafunika kuti tatenga magazi ya mwana ali yake, na kupima kalombo ka HIV (DNA/ PCR). Kupima kwa magazi kwa iyi njira ndiye kunga thandize kuziba kuti mwana ali na kalombo ka HIV kapena alibe. Iyi njira (yaba WHO)

bamaisebezetsa ngati mwana abwela ku chipatala niodwara, nipamene banga mupime chabe nakuona ngati ali nakalombo ka HIV ndikumuyambisa mankwara.

Njira yamaphunziro

Aya maphunziro niya bana bali na myezi ikalibe kufikapa khumi, isanu ndi itatu (<18), bana bamene bapezeka monga bali na kalombo ka HIV chifukwa champhavu zyochoka kwa amaiao, aliku embekeza mayanko ya magadzi yayoo (PCR/ DNA) na osunga ao anavomekeza kutengako mbali mumaphunziro. Namafunso azafunsidwa na zolemba za mwana ziza gwilitwiwa nchito po peza zina zache zofunikila kwa a dotoro. Mwana azapimindwa po peza zina zache. Magazi a zatengedwa yo lingana na zipendo zibili po funa kupima zochingiliza mwana kumatende nakupaka kwa magazi. Aya magazi yamene yazatengedwa siyaza onjezedwapo pa magazi yantawi zonse. Aya magazi ya zachotsedwa mumizipe ya mumanja namu mendo, pena na muzibelo. Aya magazi yazatengendwa kamodzi chabe, koma ngati kwapezeka zina zache zovuta, kapena magazi ayuma.

Ziopsezo zingakhalepo

Kutenga magazi kuzankala kwantawi ingono kopanda chiopnezo chosaenela kumwana. Mwana azamvera kubaba kapena kuvimba pochose, kapena pamene pachosedwa magazi. Kuchoka kwamagazi kungakhalepo, koma zonse izi zika chitika zingalesedwe, monga mwamene muzibira, kuchosa magazi zimachitika muchipatala pali bana balinakalombo ka HIV nabamene balibe, nyereti iza gwililwiwa nchito kamodzi chabe.

Phindu yingakhalepo

Aya maphunziro azathandiza bana bemene bakhara mumadera mwamene mulibe zo pimira kalombo ka HIV(DNA/PCR) ndikuyamba kumwa mankwala musanga.

Njila zina mukaleka-kutengako mbali

Mwana wanu adzapitiliza kusamalidwa mwanthawi zonse. Icichiliko mukafuna kutengako mbali kapena iyai.

Chisinsi

Nkani yonse yamwana yotengako mbali mumaphunziro idzakhala yachisinsi. Mwana azadzibika ndi nambala chabe. Koma nkhani yamwana yakuthengako mbali mumaphunziro inga onedwe ndi bungwe la University ku Alabama ku Birmingham, la USA Institutional Review Board (IRB), la Zambian Research Ethics Committee, la University la Zambia Biomedical Research Ethics Committee, bungwe la malamulo (ngati linga funidwe) ndi onse ola nganila kuti malamulo na zones zofunika za samalidwa pamodzi ndi bungwe la USA Office la Human Research Protections (OHRP).

Kusatengako mbali kapena kulekeza kopanda chiyopyezyo

Kutengako mbali kwanu mumaphunziro siko kakamiza. Ngati mwasankha kusatengako mbali kapena kusapitiriza ndi maphunziro sidzidzakhudza ku chisamalilo chaumoyo wa mwana wanu chimene alikutenga chili chonse.

Malipilo po tengekombali

Simudzalipila chili chonse ngati mwana wanu atengakombali mumamphuziro.

Kodi ndidzalipilidwa

Kulibe kulipilidwa ngati mwana wanu atengeko mbali mumamphuziro.

Malipilo chifukwa cho pwetekedwa pa kufufuza

Sichiyembekezeka kuti mwana wanu anga pwetekedwe chifukwa chakutengako mbali mumaphunziro. A sukulu lamaphunziro ya pamwamba ya Alabama ku Birmingham (UAB) sanapange makonzedwe ya ndalama yali yonse ukulipilani ngati mwapwetekedwa chifukwa cha kufufuza. Koma a bungwe la kuyanganira pa matenda yakuyambula ndi kufufuza mu Zambia (CIDRZ), adzapanga makhonzedwe yamalipilo yothandiza pa chisamalilo cha mwana ngati kupwetekedwa kwapedzeka.

Mphamvu zanu za malamulo

Simuzataya mphamvu zanu za malamulo po vemekeza kutengako mbali mu maphunziro aya.

Anthu oona pa mabvuto kapene mafunso

Ngati muli ndi mabvuto kapena mafunso pa maphunziro kuli anthu amene mungaone. Olimbitsa ndi anchito ena apa chipatala angakuthandizeni inu kuona munthu angayankhe mafuso amene muli nao:

Pa mafunso amaphunziro na zopwetekedwa pa kufufuza mungaone:-
Dr Kunda Mutesu – Kapembwa
Department paediatrics and child health,
University Teaching Hospital
Private Bag RW1X,
Lusaka
Zambia
Mobile: 0955758922

Paza mafunso pa ufulu wanu ngati otengako mbali mukufufuza:-
The Secretary,
Research Ethics Committee
Ridgeway Campus
P.O.Box 50110
Lusaka
Zambia.
Tel: 260-211-256-067

Kuvomerezana

Andi pempa paza mwana wanga/ osunga wanga kutengamo mbali mumaphunziro pazoziba njira yamene isebenzesa chipani chamene chiyanganila pa za umoyo wa bantu pano paziko lapansi igwililwidwa nchito ndi (WHO). Pofufuza njira moziwira mwana ngati ali na kalombo ka HIV amene ali ndi myezi ikalibe kufika khumi, isanu ndi itatu (<18). Zonse pali zamaphunziro aya atanthauzila namafunso yanga aniyankha mokwana. Niziba kuti ngati sinifuna kupitiriza maphunziro aya ningalekezekopanda bvuto.

Narandira pepala ya chivomerekezo yo sainiwa.

Dzina la ovomereza	**Tsiku /Sainani**
……	……
……	……

Osaphunzira zindikizani ndi chala

Dzina la mboni	**Tsiku/ Sainani**
……	……
……	……

Dzina la ofufusa	**Tsiku/ Sainani**
……	……
……	……

APPENDIX 4.

BEMBA CONSENT FORM

Ipepala lya kusuminishanya

Icishite

Ukufwailisha ukukalamba uku bomfya inshila ya balolekesha pa lwa bumi pano pesamba lya calo (WHO) ukubomfya inshila ya kutontonkanya ukupima umwana onse uwafyalwa kuli namayo onse uuli nakashishi ka HIV, ukwambilafye pakufyalwa ukufika pa myeshi ikumi limo nacine konse konse(<18), ku mwana onse ngaba muteka ku chipatala cikalamba icabana ku UTH.

UAB IRB Protocol Number: X090306005

Kafwailisha: Nine shing`anga Kunda Mutesu-Kapembwa

Akabungwe kafwilisha no lupiya: NIH/Fogarty

Icakubala

Ine nine shing`anga wa bana, kabili mbombela ku bana. Elyo nefindecita ifya kufwailisha fikangafwako palwa masambililo yandi. Mwishibe ukuti uku sendamo ulubali muli aya amasambililo tekwa kupatikisha iyo.

Umulandu wa aya amasambililo

Mwaipushiwa ukuti umwana wenu asendemo ulubali muli aya amasambililo. Umulandu wama sambililo aya, kufwailisha, ngacakuti inshila iibofya aka kabungwe akalolekesha pafya bumi pano isonde (WHO) mufyalo ifibusu nga zambia, ngakuti ya afwilisha umwana uli nakashishi ka HIV ukwamba bwangu umuti, maka maka mubana abashilakwanisha imyeshi yakufyalwa ikumi limo na cine konse-konse (<18), kabili bekala munchende umushaba ifya kubomfya ukupima akashishi ka HIV. Abana abafyalwa kuli namayo uwakwata akashishi ka HIV, kabili ilingi line balamoneka kwati nabakwata akashishi ka HIV pamulandu wamaka bafumya kuli banyinabo. Ici tacilanga ukuti umwana nakwata akashishi ka HIV nakalya, cifwaikafye ukubomfya ifibombelo ifya yana (DNA/PCR). Iyi nshila ya ba WHO ba ibomfyafye nga cakuti umwana aisa ku

Chipatala nalwala, elyo bengamupemafye, ukuti beshibe nganakwata akashishi ka HIV nokumubika pa muti.

Ifyakukonka Mumasambililo

Ayamasambililo yakuminefye kubana abashilakumanya imyeshi yakufyalwa ikumi limo na cine konse-konse (<18), abakakwata amaka yakashishi ka HIV ukufumya kuli banyinabo, elyo balelolela amasuko yamulopa ayaile mukupimwa akashishi ka HIV(DNA/PCR), nabo ababasunga balisuminisha ukusendamo ulubali muli aya amasambililo. Mukepushiwako ifipusho, nefyo bashing`anga basangile nafyo tukalabomfwa. Elyo nomba no mwana akapimwa, neci cikatwafwilishako ukulundilapo fimbi ifyo tukasanga. Tukala bulako ifi pendwa fibili ifya mulopa nokupimamo ifitucingilila mumubili, nokwishiba ubwingi bwa mulopa. Uyu mulopa tawakacile pali ulya umulopa bafumya mu chipatala lyonse iyo. Uyu mulopa tukafumya mumishipa yakumaboko nangu kumolu, kano fye ngacashupa elyo tukalafumya mumatanta. Uyu mulopa ukalasendwa umukufye umo, kanofwe ngapaba ukulufyanya pakusende umulopa nangu umulopa nga wauma.

Ifibi finga cetekelwa

Ukufumya kwa mulopa kukala citwafwe panshita inono kabili tulecetekela ukuti tekuti kulete ubusanso pa mwana. Umwana akala umfwafye ubukali panono nangu ukufimba panchende epo inshindano ikalatungwa. Nokusuma kwamulopa kuti kwabapo, lelo tukala eesha namaka yonse ukulesha ici, kabili ngefyo mwaishiba, ukufumya kwa mulopa kulacitwa mu fipatala pabana abo bali nakashishi ka HIV nabo abafyalwa kuli namayo uyo uusha kwata akashishi ka HIV. Inshindano tukalabomfya umukumo no kuposa.

Ubukumo

Ifi kasangwa muli aya masambililo, fikafwilishako abana abali muchende umushaba ifya kupimina akashishi ka HIV (DNA/PCR) ukwamba bwangu ukuba pa muti.

Inshila shimbi isha kusendamo ulubali

Umwana wenu kuti apitilisha ukundapwa ukukonka nobwanshiko bwa pa chipatala, nangu nga mwasala ukusendamo ulubali muli aya amasambililo.

Inkama

Ilyashi likasendwa palwa kufwailisha likasungwa mu nkama. Nelyashi lya ulesendamo ulubali likasungwa ukwabula ishina lyakwe. Ake shibikwafye ne nambala, elyo ilyashi lya pa mwana wenu talyakasokololwe. Lelo ilyashi lya kufwailisha lya pa mwana wenu kuti limbi lyasokololwa nefiputulwa fya; ba University baku Alabama ku Birmingham, USA Institutional Review Board (IRB), ba Zambian Research Ethics Committee, ba University baku Zambia Biomedical Research Ethics committee, neciputulwa icipela insambu, nabo bonse abamona ukuti ubwanshiko na mafunde palwa kufwailisha nafikonkwa, pamo naba USA Office ba Human Research Protections (OHRP).

Ukukana nangu ukufumamo ukwabula ubwafya

Ukusendako ulubali muli aya amasambililo tekwakupatikisha iyo. Nga mwakana ukusendako ulubali nangu mwafwaya ukuleka aya amasambililo panshita ili yonse, tacakakume kukutangatwa palwa bumi bwa mwana uko apokelelwa munshila ili yonse.

Amalipilo pa kusendamo ulubali

Takwakabe ukumilipisha nga mwasala ukuti umwana wenu asendemo ulubali mumasambililo aya.

Bushe bakandipila

Ukusendamo ulubali kwa mwana wenu mu masambililo aya takwakabe amalipilo.

Ukulipilwa palwa kucenwa ukukumine kukufwailisha.

Tacilepala kwati umwana wenu kuti acenekwa pa mulandu wakusendemo ulubali mumasambililo aya. Ba University baku Alabama ku Birmingham (UAB), tababikako indalama shakulipila, ngacakuti ukucenwa kwacitika ukufuma kukufwailisha, lelo iciputulwa cifwailisha pa malwele ya lwambu mu Zambia (CIDRZ) bakapekanya ukulipila indalama shonse isho bakamupingula pa ku mundapa pamulandu wakucenekwa uku kumine ku kufwailisha. Ngacakuti umwana wenu acenekwa pa mulandu wakusendamo ulubali mu masambililo aya, baka mundapa ukuli ngana no bwanshiko bwa mufipatala fya mu Zambia.

Insambu shenu isha mafunde

Tamulefumyapo insambu shenu isha mafunde isho mwakwata nga mwa saina ipepala lya kusuminishanya.

Abantu bakumona pa mafya nangula amepusho

Nga namukwata amafya nangu amepusho palwa masambililo kuli abantu abakumona, Ba kansela elyo nababomfi ba pachipatala kuti bamwafwilisha ukumona abantu abalingile ukwasuka amepusho yenu.

Pa mepusho ya pa masambililo nangu ukucenwa ukukumine kukufwailisha moneni;

Dr Kunda Mutesu Kapembwa
Department of Paediatrics and Child Health
University Teaching Hospital
Private Bag RW1X
LUSAKA
ZAMBIA
Mobile: 0955758922

Pa mepusho ya nsambu shenu ngo ulesendamo ulubali;

Ba Secretary
Research Ethics Committee
Ridgeway Campus
P.O.Box 50110
LUSAKA
ZAMBIA
TEL: 260-211-256-067

Ukusuminishanya

Naipushiwa palwa mwana wandi nangu umusungwa wandi ukusendamo ulubali mumasambililo palwa kwishiba inshila ibomfya ici putulwa icilolekesha palwa bumi (WHO) pakusanga inshila iyakwishiba umwana ngali na kashishi ka HIV mubana abali nemyeshi ishilakwana ikumi limo na cine konse-konse (<18).

Fyonse palwa masambililo naba nondolwela, namepusho nabasuka ukulingana nomonacilafwaila. Ningumfwikisha ukuti ndinensambu shakuleka aya masambililo panshita ili yonse ukwabula ukusanga ubwafya ubuli bonse. Kabili nabampela ipepala ilyotusuminishenye ilyasainwa.

Ishina lya umusunga ubushiku/ saina

…… …….

Tasambilila (afwatike icikumo)

……...

Ishina lya kwa kambone ubushiku/saina

…… …….

Ishina lya kwa kafwailisha ubushiku/ saina

…….. ……

ANNEX A.

WHO PRESUMPTIVE DIAGNOSTIC CRITERIA

Clinical criteria for presumptive diagnosis of severe HIV disease in infants and children less than 18 months of age requiring ART in situations where virological testing is not available.

A presumptive diagnosis of severe HIV disease should be made if;

- The infant is confirmed HIV antibody positive
  and
- Diagnosis of any AIDS- indicator (conditions) can be made;
  or
- The infant is symptomatic with two or more of the following;
  - oral thrush
  - severe pneumonia
  - severe sepsis

Other factors that support the diagnosis of severe HIV disease in an HIV seropositive infant include;

- Recent HIV-relative maternal death; or advanced HIV disease in the mother;
- CD4% <20

Confirmation of the diagnosis of HIV infections should be sought as soon as possible.

ANNEX B.

IMCI CASE DEFINITIONS

IMCI General Danger Signs

Lethargic or unconscious, not able to drink or breastfeed, vomiting and presence or history of convulsions during current illness.

Severe Pneumonia

Cough or difficulties in breathing in a child with chest in-drawing, stridor or any of the IMCI general danger signs.

Severe Sepsis

Fever (37.5oC) or low body temperature (35.5oC) in young infant with any severe sign such as; fast breathing, chest in-drawing, bulging fontanelle, lethargy, reduced movement, not feeding or sucking breast milk, convulsions etc.

Oral thrush

Creamy white to yellow soft small plaques on red or normally coloured mucosa which can often be scraped off (pseudo membranous), or red patches on tongue, palate or lining of mouth, usually painful or tender. Not responding to topical antifungal treatment.

ANNEX C.

DEVELOPMENTAL MILESTONES

Surveillance tool for children 0-18 months from Paediatric Centre of Excellence in HIV/AIDS Management

	Interpersonal development	Motor development	Language development	Self help development
0-4 months	Looks at face. Begins to develop social smile. Responds to loud noises. Looks at own hand.	Brings thumb and/ or fist to mouth.	Produces these sounds: ooo/aaaah. Responds (Alerts) to bell.	
4-6 months	Smiles responsively. Responds to friendly, angry tones. Looks at interesting objects and surroundings.	Reaches for objects. Grasps. Puts objects in month. **Sits with support.**	Squeals. Laughs. **Turns to rattling sound.**	**Tries to hold feeding bottle or breast with hands while drinking.**
6-9 months	Responds differently to strangers. **Responds to other people's expressions of emotion.**	Sits without support. Can bear weight when put in a standing position (can't stand alone). Crawls. Transfers objects from one hand to the other.	**Babbles.**	**Feeds self biscuits.**
9-12 months	Pays attention to own name. Responds differently to strangers than to familiar people.	Drops and picks up toys. **Stands holding on to a supporting object.**	**Holds cup with both hands; drinks with assistance.**	

	Interpersonal development	Motor development	Language development	Self help development
15 months	Indicates some desires or needs by pointing Hugs parents	Able to walk without support Craws up stairs	Jargon ,follows simple commands May name a familiar object (e.g ball)	Makes a line with a crayon
18 months		Runs stiffly, sits on small chair, walk upstairs with one hand held, explores drawers and waste baskets	Speaks at least 10 words, names pictures Identifies one or parts of the body	Feeds self, seeks help when in trouble, may complain when wet or soiled