DECLARATION

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part to any forum or University other than the University of Zambia.

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By

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APPROVAL

This dissertation of Dr. Catherine Mupela Chunda has been approved as fulfilling the requirement for the award of the Degree of Master of Medicine in Paediatrics and Child Health by the University of Zambia.

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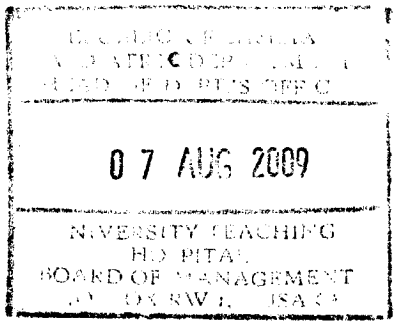

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ABSTRACT

Background

Zambia is a resource constrained country with a Human Immunodeficiency Virus (HIV) prevalence of 16% among adults aged 15-49 years. The HIV sero prevalence in urban antenatal clinics is approximately 25% and it is estimated that 28,000 infants are born with HIV infection annually.[1] Prevalence among antenatal attendees in Lusaka is about 20%, and one out of five infants born in Lusaka is perinatally HIV exposed. The University Teaching Hospital (U.T.H) continues to see an increase in the number of HIV infected infants and young children. In the context of the global effort to scale up paediatric HIV care, the Zambian government through the Ministry of Health introduced "Provider initiated HIV testing and counselling" (PITC) in all health facilities in 2006. UTH, the largest tertiary institution in Zambia and its Department of Paediatrics has pioneered this initiative. Health care providers are offering all paediatric in-patients PITC. Results since 2006 indicate over 85% acceptance among care givers. [1] A non-invasive, rapid HIV test could further increase accessibility of HIV diagnosis among children in resource-poor settings.

The use of oral fluid to screen for HIV infection in adults is well established. However, few performance data exist among infants and young children worldwide. The objective of this study was to determine the sensitivity and specificity of an oral fluid HIV test compared to standard blood-based HIV rapid tests, and to assess the acceptability of using oral fluid testing among caregivers of children younger than age 18 months.

Methods: Children aged 1 day to 18 months admitted to the U.T.H Department of Paediatrics in Lusaka, Zambia between December 2006 and March 2007 were recruited for this study. Oral fluid and finger prick blood specimens were collected and the serostatus of the children was established using a serial algorithm composed of: Abbott Determine HIV-1/2 and Genie II HIV-1/2 with discordant results resolved using a tie breaker, Bionor. The characteristic of the OraQuick rapid HIV-1/2 assay test was assessed against the established serostatus of the specimens.

Results: Of the 1000 children tested in the study, 270 (27 %) were found to have HIV antibodies. The oral fluid, OraQuick detected HIV antibodies among children less than 18 months of age with 100% sensitivity (95% CI: 97.7-99.9) and 98.9% specificity (95%

CI: 97.2-99.2); with a positive predictive value of 97.1% (95% CI: 92.8-97.9). Caregiver preference for oral fluid testing increased from 1% before the start of the testing to 81% after the testing experience ($p<0.001$).

Conclusions: The oral fluid rapid test was found to be a highly accurate and acceptable method of screening for HIV antibodies in children younger than 18 months of age. This test has the potential to increase acceptability of HIV screening in infants and young children in resource-limited settings. The high rate of detection of HIV antibodies in infants also underscores the need to expand access to viral detection assays for diagnosis of HIV infection infants.

DEDICATIONS

To my husband, Liyoka, who has always supported me. I can always count on you; my children, Shoyo, Mupela and Nkomeshya you always challenge and inspire me to aim high.

To my parents, who taught me the value of education; my brothers and sisters, who always look up to me.

To my supervisor and my mentor, Dr. Chipepo Kankasa, who inspires me and I continue looking up to you for guidance.

ACKNOWLEDGEMENT

I am greatly indebted to the two organizations that sponsored the study: Financial support was provided by Thrasher Research Funds; and the OraQuick kits were provided by CDC-Zambia.

My deep gratitude to my Supervisor, Dr. C. Kankasa, my Co-supervisor, Dr. Katepa-Bwalya for their patient supervision, teaching and guidance throughout the study.

The OraQuick team, for their high motivation and tireless effort to complete the study within schedule;

- The Project Assistant: Mrs. Febby Kawamya, for her very efficient supervision
- The Nurse-counselors: Miss. Nora Nyirenda; Mrs. Ivy Muzyamba; Mrs. Ruth Katuta and Mrs. Eneless Muzyamba
- Laboratory staff: Mr. Paul Chilunje
- Data Entry clerk: Miss Nkandu Kawamya
- Data Manager: Mr. Katai Chola
- Other assistance:Mrs.Erica Spielman, for her assistance with the OraQuick training and co-ordination with CDC-Zambia
- Financial management: Dr Tendai Msoka and Mr. Jack Chewe.

To the many people that assisted with the data analysis and with the corrections of the dissertation, my special thank you:

Dr. Marc Bulterys, Dr. Ann Chao, Dr. Somwe wa Somwe, Dr. Mary Ngoma, Dr. Veronica Mulenga, Dr. Mutinta Nalubamba Phiri, Dr. Wilbroad Mutale and Dr. Peter Chungulo.

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ACRONYMS AND ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral medicine
bDNA	branched chain DNA
CD4	human T helper cells expressing CD4 antigens (T helper Cells)
DBS	dried blood spot
DNA	deoxyribonucleic acid
EIA	enzyme immunoassay
ELISA	enzyme-linked immunoassay
FDA	food and drug administration
GACELISA	IgG antibody capture enzyme-linked immunosorbent assay
GACRIA	IgG antibody capture radioimmunoassay
GENIE 11	is a rapid enzyme immunoassay for the qualitative detection of antibodies to HIV virus
HIV	human immunodeficiency virus
HAART	highly active antiretroviral therapy
ICD	immune complex dissociation
Ig	immunoglobulin
IMCI	integrated management of childhood illnesses
MIRIAD	mother infant rapid intervention at delivery
(P)MTCT	(prevention of) mother to child transmission
NASBA	nucleic acid sequence based amplification
NPV	negative predictive value
OMT	oral mucosal transudate
PCR	polymerase chain reaction
PPV	positive predictive value
P24	a soluble antigen produced by HIV
RNA	ribonucleic acid
RT	reverse transcriptase
Se	sensitivity
Sp	specificity
WHO	world health organization

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Testing persons for HIV is important in controlling the impact of the HIV and Acquired Immunodeficiency Syndrome (AIDS) epidemic. It allows for the diagnosis and counselling of HIV-infected persons and facilitates the implementation of prevention and treatment strategies. Examples of such strategies include: administration of prophylactic antiretroviral therapy to mothers and their babies for the prevention of mother-to-child transmission of HIV; initiation of prophylactic cotrimoxazole to perinatally exposed and infected infants and the initiation of antiretroviral therapy in those that meet the criteria for treatment. In order to benefit from such therapy, HIV-infected persons must be timely identified through HIV testing.

Serological identification of antibodies to HIV in blood is the most widely used method to screen and confirm HIV infection. The HIV, Enzyme-Linked Immunosorbent Assay (HIV ELISA) and rapid antibody tests are the most widely available and used tests. In children below 18 months, the antibody test only defines risk of infection. Despite their limitation, the tests provide (or exclude) evidence of exposure and are important for screening HIV in this age group. In adults and children above 18 months, a definitive diagnosis of HIV infection can be made using two or three rapid antibody tests. Advances in technology have led to the development of a wide variety of rapid HIV tests, including oral based antibody tests.[2-5]

Oral fluid samples have been used as an alternative to blood samples for the detection of anti-HIV antibodies. The fluid used contains crevicular fluid from capillaries beneath the tooth-gum margin thus is a transudate of blood. It include the same fluid (plasma) that is used for testing with serum-based tests. The concentration of antibodies in oral fluid (true saliva) is about 1/400 of that in plasma, because of the dilutional effect of fluid from the salivary glands. This necessitates extremely sensitive tests that are able to detect very small quantities of antibody.[4]

This testing technology to detect low quantities of anti-HIV antibodies has been introduced. The test, called OraQuick Advanced Rapid HIV 1/2, is a combination of a collecting and testing device. It consists of an absorbent pad on a stick coupled to a lateral flow testing device. It is used to swab once around the gums and is placed in a vial of a buffer solution. Following 20 minute incubation, the results are read like other lateral flow rapid tests. A control line is also included. The manufacturer claims a 100% sensitivity and specificity that is equivalent to ELISA HIV tests. This device can also be used for testing serum, whole venipuncture blood and blood collected via fingerprick, thereby giving flexibility for the different testing situations. Other advantages of Oraquick include: the ease of collection (non-invasive); group collection; collection from persons in whom blood is very difficult to draw (infants); an increase in collection compliance and reduced hazard risk to the health worker collecting specimens.[6,7]

Zambia like most countries in Sub-Saharan Africa has a high HIV sero-prevalence rate among the adults. This is reflected by the growing paediatric HIV population in sub-Saharan Africa. Mother to child transmission is by far the largest source of HIV infection in children less than 15 years and is estimated to contribute to 90% of paediatric infections.[8] The HIV prevalence rates among antenatal mothers ranges from 6.8-14% in rural areas to 15-37% in urban areas. The mother to child transmission rates of HIV without interventions is estimated to be 39.5%.[9,10] Health institutions in several parts of the country have limited HIV laboratory resources and trained personnel. Only two out of the nine Provinces in the country have virology testing facilities for confirmation of HIV in children less than 18 months old. Therefore repeated blood based antibody tests have to be done if virology testing is not available.

The Paediatrics Department of the University Teaching Hospital (U.T.H), Lusaka, Zambia, at any given time has 40% of in-patients with HIV/AIDS related illness.[11] Therefore more widespread HIV testing is needed. This will enable early identification of children who are exposed and infected with HIV. It will also facilitate timely institution of preventive measures such as antiretroviral (ARV) and cotrimoxazole prophylaxis and initiation of ARV treatment. However, the current blood testing makes such widespread HIV testing nonviable in this challenging population of children.

1.2 JUSTIFICATION OF THE STUDY

Zambia has a high paediatric HIV prevalence and therefore needs to screen more children for the infection. Children less than 18 months comprise the majority (60%) of paediatric in-patients at the Department of Paediatrics and Child Health, U.T.H, Zambia. There is need for a non-invasive, rapid, on-site screening tool for HIV since screening and confirmation of HIV infection is very difficult in children in this age group. Infants and young children would benefit from the advantages of using an oral based HIV test, such as OraQuick. However, oral fluid testing as an alternative to blood testing for HIV screening has not been validated in children below 12 months of age. This studys' aim was to evaluate the feasibility and acceptability of using OraQuick as a screening test for HIV in children below 18 months of age.

CHAPTER 2

OBJECTIVES

2.1 MAIN OBJECTIVE

To determine the feasibility and acceptability of using OraQuick Rapid HIV 1/2 antibody test for screening HIV in children less than 18 months of age.

2.2 SPECIFIC OBJECTIVES

1. Assess accuracy of OraQuick in detecting HIV antibodies in children less than 18 months.

2. Assess the acceptability of using oral fluid to screen for HIV in the children's caretakers

CHAPTER 3

LITERATURE REVIEW

3.1 DIAGNOSIS OF HIV INFECTION

Accurate diagnosis of HIV infection depends on laboratory tests which provide suggestive and/ or confirmatory evidence of HIV infection.[3] These tests are of two types namely virological and serological tests.

The virological tests detect the HIV virus itself or components of the virus. They are very important for confirmation of HIV infection in children less than 18 months. Some of these tests in current use are: HIV DNA PCR (deoxyribonucleic acid, polymerase chain reaction) assays, RNA (ribonucleic acid) assays including viral load, and HIV immune complex-dissociated p24 antigen assays.[2,3,11]

The serological tests detect HIV antibodies; which are proteins produced in response to HIV infection. The antibodies detected are of the Immunoglobulin G (IgG) class. The tests are used for screening HIV in children less than 18 months and confirmation of infection in children above 18 months of age. Examples of serological tests include: HIV ELISA, Rapid test and Western blot. HIV ELISA detects IgG antibody to HIV and are of two types; Standard long ELISA and rapid tests. Western blot detects antibodies to HIV proteins (p24, glycoprotein (gp) 41, gp120, gp160).[2,11]

3.1.1 VIROLOGIC TESTS

3.1.1.1 HIV Immune Complex Dissociated p24 Antigen Assays

The p24 protein (antigen) is from the core protein of the HIV virus. Detection of p24 antigen is definitive evidence of HIV infection.[2] It can be used for diagnosis in children less than 18 months of age.

a) STANDARD ASSAY

The p24 antigen in the serum of infants is bound to maternal HIV antibody. This test is insensitive for the detection of HIV infection in infants since it only detects free antigens and less than 20% have detectable p24 antigen at 1-6 months of age.[2]

b) ICH-HIV-P24 ASSAY

In this assay, acid hydrolysis is used to disrupt antigen-antibody complexes in serum, the sensitivity of p24 antigen detection can be increased, and this assay may be a tool for early diagnosis. 100% are positive by 1-3 months of age.[2]

3.1.1.2 HIV DNA PCR

DNA PCR assays amplify the HIV pro-viral DNA sequence within mononuclear cells present in peripheral blood. The results of such assays are the accepted standard for diagnosis of HIV infection during infancy and early childhood.[2,11]

The sensitivity of HIV DNA PCR is low during the first 1 to 2 weeks of life because this test is not able to detect very low levels of HIV DNA in babies infected a few minutes/hours/days earlier, during delivery and early breast-feeding. After 4 to 6 weeks of life, the sensitivity and specificity of HIV DNA PCR test approaches 100% for children infected in utero or intrapartum. DNA remains positive in children on treatment and therefore is reliable even in the context of maternal prophylaxis or treatment. Earlier disadvantages to HIV DNA PCR included less sensitivity in detecting non-B subtypes, but the new version has incorporated primers that offer increased sensitivity for non-B strains. Other disadvantages are that HIV DNA PCR is expensive since it requires specialized laboratory equipment and skilled personnel and also samples may become contaminated with HIV DNA from other sources giving false positive results.

New technologies, such as real-time PCR could provide a good alternative. This technique addresses amplification and detection of nucleic acid in one step. Advantages of this technology are increased sensitivity and decreased risk of contamination. World Health Organisation (WHO) guidelines report that real time PCR is rapid, simple, cheap and adaptable to the different clades of HIV and has recommended it as the preferred method.[1]

3.1.1.3 HIV RNA Assays

HIV RNA tests have been found to be as sensitive and specific as HIV DNA assays. They detect viral RNA in plasma and other body fluids using a variety of approaches including reverse transcriptase (RT), branched chain DNA (bDNA), and nucleic acid sequence based amplification (NASBA). RNA assays have a faster turnaround time and require smaller blood volumes. This assay is also more sensitive for early detection of infection (first 2 months of life) than HIV DNA PCR tests.[3]

Quantitative RNA (viral load) is used to determine the risk of HIV disease progression and to guide decision for initiation of ART. This test however is expensive.

3.1.2 SEROLOGIC/ANTIBODY TESTS

Serologic, also called antibody tests are HIV diagnostic tests that can provide reliable evidence of HIV infection in adults and children who are older than 18 months. However these tests are less reliable in infants aged less than 18 months because they may still be carrying HIV-specific antibodies acquired from the mother in utero. The time it takes for an HIV- positive mother's maternal antibodies to be eliminated from an infants system (sero-conversion) varies. The majority of uninfected non-breast-fed children will seroconvert by age 15 months, but a similar percentage (ranging from a low of 1% to a high of 18% in various studies) will not revert until age 18 months.[2]

3.1.2.1 STANDARD LONG ELISA TEST

The ELISA method in a micro plate format was the commonest screening test for IgG HIV antibodies before the introduction of rapid tests. The technique uses HIV antigens derived from HIV grown in human T lymphocytes or recombinant proteins immobilized on beads or micro plate wells. A patient's serum is incubated with the immobilized antigens. Subsequently, an enzyme-labelled antibody specific for human immunoglobulin is added. Detection of the enzyme-labelled antibody occurs by the addition of a substrate that yields a coloured reaction product. Competitive ELISA's and double-antigen assays are two modifications also in use.[12]

3.1.2.2 RAPID TESTS ASSAYS

Most rapid assays are in kit form, including all necessary reagents and requiring no specialized equipment. The three most common assay formats are:

a. Particle agglutination
b. Immunoconcentration
c. Immunochromatography

a) PARTICLE AGGLUTINATION

Particle agglutination typically requires 10-60 minutes or more to obtain a result. Cross-linking occurs, resulting in agglutination when a specimen containing HIV antibodies is mixed with latex particles coated with HIV antigen. Results are interpreted visually.[12]

b) IMMUNOCONCENTRATION (flow through)

These devices employ solid-phase capture technology, which involves mobilization of HIV antigens on a porous membrane. The specimen flows through the membrane and is absorbed into an absorbent pad. Some tests allow the differentiation of HIV-1 from HIV-2 by applying antigens from these viruses to the different sites of the membranes.[12]

c) IMMUNOCHROMATOGRAPHY (lateral flow)

This is the most recent development. It incorporates both reagent and signal reagent into a nitrocellulose strip. Many lateral flows only require a single step. The specimen is applied to an absorbent pad or diluted in a vial of buffer into which the test device is inserted. The specimen migrates through the strip and combines with the signal reagent. A positive reaction results in a visual line on the membrane where HIV antigens have been applied. A procedural control is usually applied to the strip beyond the HIV-antigen line. Some tests apply HIV-1 and HIV-2 antigens in different locations and allow differentiation of antibodies to these two viruses. Many of these assays can use whole blood, serum, plasma, finger-stick blood specimens and oral fluids.[12]

A visual line at both the test and control sites indicates a positive test result, whilst a line only at the control site indicates a negative result. The absence of a line at the control site means an invalid test.

3.2 ADVANTAGES OF RAPID HIV TESTS [3]

FEASIBILITY

In countries with a limited laboratory infrastructure, the use of HIV rapid testing algorithms has been more feasible and as effective as ELISA/Western Blot algorithms.

DECENTRALIZATION OF HIV TESTING AND COUNSELLING

This is of major importance because it allows HIV testing and counselling to be decentralized to community services away from major urban centres. Rapid tests are especially suited for use in rural settings. A key advantage of using rapid tests is that the reliance on laboratory services for obtaining test results is dramatically reduced only if the minimum standards for ensuring the quality of test procedures and record keeping are observed. Available data show that there is a high degree of acceptance of decentralized services by clients. Moreover, the speed of obtaining the test results has led to their greatly increased uptake.

ACCEPTABILITY OF HIV TESTING AND COUNSELLING

ACCEPTABILITY TO CLIENTS

In developed countries a large proportion of people who are tested for HIV in clinical settings or at voluntary counselling and testing sites do not return for their test results.[13] This wastes both financial and human resources and means that some people who test positive do not benefit from treatment, care and prevention options. Many testing and counselling sites have reported an increased demand after rapid testing was introduced. In studies at HIV testing and counselling sites in resource-constrained settings the proportion of patients who received post-test counselling increased significantly after the introduction of rapid testing. A trial in Kenya in which women were randomly assigned to receive either rapid tests or ELISA's showed that the former significantly increased the proportion of clients receiving test results.[13-15]

Use of rapid tests that use non-invasively collected specimens such as oral fluid has further increased acceptance of testing among high risk populations such as sex industry workers, intravenous drug users and sexually transmitted disease clinic.

ACCEPTABILITY TO COUNSELLORS

Several reports and case studies[13,14,16] have indicated that rapid tests improve the acceptability of HIV testing to both providers and clients.

SHORT TIME TO OBTAIN TEST RESULT

Most rapid tests provide results within 10–30 minutes.

REDUCED COST

In general, rapid tests are slightly more expensive than ELISA's but do not require the initial investment in equipment and ongoing operational expenses. In practice the reagent cost per test result is considerably higher with ELISA's than with rapid tests, unless all 40–90 reagent wells of the former are used. The rapid test algorithm leads to a greater proportion of clients receiving their test results, reduced wastage of test kits and increased efficiency. In comparison with other testing strategies, testing algorithms based on rapid tests have a lower cost per patient receiving results.[17] Client's transportation costs and travel times are decreased, as rapid tests provide same-day results, and the costs to health services are decreased because fewer return visits to clinics are required.

EASE OF PERFORMANCE AND INTERPRETATION OF TEST RESULTS

Non-laboratory health care workers can perform most of the rapid tests after adequate basic training. This training should cover correct client identification, the performance and interpretation of the test within the specified reading time, assuring the quality of results, record-keeping, the maintenance of client confidentiality, bio-safety including safe waste disposal.[18]

MINIMAL FACILITIES FOR STORAGE AND SHELF-LIFE

Most rapid tests require no laboratory equipment and can be performed in settings with limited facilities. Many such tests do not require refrigeration and are therefore particularly suitable for remote and rural areas without a constant electricity supply. However, the temperature should not fall below 2 °C or rise above 20–30 °C, depending on the test kit used. Extreme low or high temperatures affect the quality and shelf-life of the tests.[3] Therefore, it is advisable to monitor temperature fluctuations in

storage rooms. In practice, a refrigerator or an air-conditioned room may be required in tropical climates.

FLEXIBILITY IN NUMBERS OF TESTS PERFORMED

Several rapid test kits allow the testing of single specimens whereas the design of ELISA's makes them most suitable for batch testing, i.e. at least 40–90 specimens per run. ELISA's may be suitable for settings in which a large number of tests are performed. However, at many testing and counselling sites the ability to perform single or small numbers of tests is a key advantage.

REDUCTION IN OCCUPATIONAL EXPOSURE RISK

Most occupational exposure occurs during venipuncture. The risk of such exposure is substantially reduced with finger-prick blood collection and more so with the use oral fluid specimens.

FLEXIBILITY OF TESTING SITUATIONS

The newly introduced rapid tests such as OraQuick can be used for testing oral fluid, serum, whole venipuncture and finger prick blood.

3.3 ORAL FLUID AND HIV TESTING

Oral fluids have been widely used for monitoring drugs, hormones and a variety of other molecules and chemical substances for over 50 years. In the past, the use of oral fluid has been used as a non-invasive alternative to the collection of blood for the detection of antibodies to bacteria, viral, fungal and parasitic agents.[19] Use of oral fluid for detection of HIV antibodies has been explored and is currently being used.[6,7]

Oral fluid testing for HIV antibodies was first reported by Archibald and Parry.[19] As summarized by studies with saliva conducted between 1986 and 1991, the concordance between positive serum tests and positive saliva test for the detection of HIV antibodies ranged from 70-100%. Factors attributed to the less than perfect results and thus factors that can lower test sensitivity were found to be: the type of oral fluid collected; amount of oral fluid collected; handling of sample prior to testing; the concentration of IgG and modification of testing methods to accommodate use of saliva. Current methods being used have developed specialized collecting devices that enhance the level of antibodies, particularly IgG in oral specimens. This ensures sufficient specimen volume and includes reagents to prevent microbial growth and proteolytic breakdown of antibodies.

The oral fluids that can be used include whole saliva, glandular-duct saliva and oral mucosal transudates, (OMT) which is the specimen of choice. It comprises fluid from the capillaries beneath the buccal mucosa and at the base of the crevice between the teeth and gums. These fluids not only contain secretory IgA but are rich in IgG and IgM. The immunoglobulins originate from the plasma and are passively transferred to the mouth across the mucosa and through the gingival crevices. The concentration of IgG in oral fluid is known to be 800-1000 times less than that in serum. This low concentration of IgG contributes to the reduced sensitivity observed with oral fluid specimen when tested by the current enzyme immunoassay. Therefore the device used for its collection has to be sufficiently enriched and elute the IgG antibodies. Several of these devices are commercially available for the collection of OMT. These include: Salivette; Orapette; Omni-SAL; Orasure and Foam swab.[4,19]

Screening assays that have been devised for the detection of HIV antibodies in oral fluid include: rapid tests; conventional enzyme immunoassays (EIA); IgG antibody capture radioimmunoassay (GACRIA) and the enzyme-linked immunosorbent assay

(GACELISA) optimized for the detection of HIV antibody in specimens that contain low concentrations of immunoglobulin. Western blot technique has also been devised as confirmatory testing using whole saliva and oral fluid specimens collected with various devices.[19]

ORAQUICK ADVANCED RAPID HIV-1/2 ANTIBODY TEST

OraQuick Advanced Rapid HIV type 1/2 rapid antibody test is designed as a rapid test that uses oral mucosal transudate (OMT), whole blood, serum or plasma. It was approved by the U.S. FDA in November 2002 for HIV-1/2 diagnosis by using finger-stick whole blood. Early trial of OraQuick on OMT, blood, and serum demonstrated 100% sensitivity and 99.8% specificity.[12]

BIOLOGICAL PRINCIPLES OF THE TEST

OraQuick detects gp41 immunodominant domain antibody. The antibodies tested for are of the IgG subclass since the primary humoral immune response to HIV infection involves mainly this class of antibodies.[7]

The test is a manually performed, visually read, 20 minutes immunoassay. It is used for the qualitative detection of antibodies to the HIV 1/2 in human oral fluid, whole blood obtained from a finger puncture or a venipuncture and plasma. It is comprised of a single-use test device and a single-use vial containing a pre-measured amount of a buffered developer solution. The test utilizes a proprietary lateral flow immunoassay procedure. The assay test strip contains synthetic peptides representing the HIV envelope region and a goat anti-human IgG procedural control immobilized onto a nitrocellulose membrane in the Test (T) zone and the Control(C) zone, respectively. The built-in procedural control serves to demonstrate that a specimen was added to the vial and that the fluid has migrated adequately through the test device. The intensity of the colour is not directly proportional to the amount of antibody present in the specimen. The test result is interpreted after 20 minutes, but not more than 40 minutes after the introduction of the test device into the developer solution containing the test specimen.[7]

PERFORMANCE CHARACTERISTICS OF ORAQUICK

Oraquick has demonstrated a sensitivity of 99.3% at several clinical trials using oral fluid and 99.6% using whole blood via needle prick test. The test assay has also been demonstrated to be capable of detecting seroconversion similar to currently available FDA licensed EIA's.[7]

POST-MARKETING SURVEILLANCE

Post-marketing surveillance was conducted to monitor the performance of the OraQuick test on whole blood and oral fluid. The conclusion of the surveillance was that specificity of assay performed on whole blood and oral fluid was compatible with the manufacturer's claim within the package insert. During the surveillance period, 135 724 whole blood and 26 066 oral fluid rapid tests were conducted. The median health department whole blood OraQuick specificity was 99.98% (range: 99.73-100%) and positive predictive value (PPV) was 99.24% (range: 66.67-100%); the median oral fluid specificity was 99.89% (range: 99.44-100%) and PPV was 90.00% (range: 50.00-100%). A total of 124 discordant results were reported from 68 (0.05%) whole blood and 56 (0.22%) oral fluid rapid tests. The oral fluid specificity at the site with excess oral fluid false-positive tests was 98.7% (95% confidence interval: 98.18-99.11%). The increase in false-positive tests at that site was not associated with any specific device characteristic, operator procedure or temperature condition.[20]

RAPID TESTS PERFORMANCE COMPARED

Abbott Determine, Capillus, OraQuick, Unigold and Hemastrip were evaluated to develop a rapid test based testing algorithm for Voluntary Counselling and Testing (VCT) in Ethiopia. The reported sensitivity and specificity ranged between 99% to 100%. At the end of the study, Determine, OraQuick and Unigold were recommended as screening, confirmation and tie-breaker tests respectively.[21]

In Uzbekistan, OraQuick has been recommended for use in epidemiological investigations when it yielded 95% sensitivity and 100% specificity compared to the ELISA serum based test.[22]

In Ivory Coast sensitivity and specificity of four rapid tests were evaluated in order to develop a serologic testing algorithm for HIV-1 and HIV-2 infection. Abbott Determine HIV-1/2, Capillus HIV-1/2, HIV-SPOT and Genie II HIV-1/2 were used. All the four assays were 100% sensitive and specificities ranged from 99.4%-100%. The subsequent parallel and serial testing algorithms evaluated were 100% sensitive and specific. This study was carried out on pregnant women attending antenatal clinic.[23]

Abbott Determine and Genie II were evaluated in a serial testing algorithm to determine their ability to diagnose HIV and differentiate HIV -1 from HIV- 2. Participants for this study were recruited from pregnant women in the PMTCT programme in Abidjan, Ivory Coast. The sensitivities yielded were 100% for Abbott Determine and 99.1% for Genie II. The specificities were 98.4% for Abbott Determine and 100% for Genie II.[24]

3.4 APPLICATION OF ORAL-BASED HIV ANTIBODY TESTS IN VARIOUS SETTINGS

In four separate studies in the U.S, the accuracy of the rapid test performance on whole blood and oral fluid specimens were compared with the results of conventional HIV tests in different settings were the test is likely to be used. Oral fluid and whole blood from persons of unknown HIV status recruited from clinics, labour and delivery units and outreach venues were tested with OraQuick. OraQuick sensitivity was 99.7% with whole blood and 99.1% with oral fluid. Specificity was 99.9% with whole blood and 99.6% with oral fluid. A cluster of 16 false-positive oral fluid tests occurred in one study which also reported a lower specificity than in the other three studies.[25]

The sudden increase in false positive results occurring in performance studies of OraQuick, oral fluid tests in Minnesota prompted a field investigation. The study reviewed performance study data on oral fluid and whole blood OraQuick rapid HIV test device lots and expiry date. They also assessed test performance and interpretation with oral fluid and whole blood specimens by operators who reported false positive results. The field investigation did not identify a cause to increase in false positive oral fluid results. The incidence study in the nine U.S cities detected no false positive result. These findings suggested this was an isolated cluster; the test's overall performance was as specified by the manufacturers.[26]

Field evaluation of diagnostic accuracy, client preference and feasibility for OraQuick rapid HIV-1/2 test in a rural hospital in India was assessed. A cross-sectional, hospital-based study was conducted in 450 consenting adult participants with suspected HIV infection. Both oral fluid and finger-stick testing were carried out on OraQuick. Confirmation ELISA and western blot were the reference standard. Oraquick test on oral fluid had sensitivity and specificity of 100% compared to 100% sensitivity and 99.7% specificity on finger-stick. The oral fluid based test was preferred by 87% of participants for first line testing and 60% for repeat testing. OraQuick was recommended to work well in rural resource limited settings.[27]

Rapid HIV antibody tests (RT) now permit HIV screening in settings where laboratory personnel may not be available. One study assessed the ability of 99 individuals with no laboratory experience to conduct two rapid tests, OraQuick and Hema-Strip. The study results were compared with those generated by laboratory professionals. All participants received written instructions and one-half also received a short demonstration. Error rates ranged from 2.1% to 4.6% with or without a demonstration. However, the number of invalid tests was greatly reduced when participants received a demonstration. Appropriate rapid test training for non-laboratorians and continued monitoring of HIV rapid test performance in non-laboratory settings was recommended after this study.[18]

ORAL FLUID HIV TESTING IN CHILDREN

Sherman G et al in a prospective, longitudinal study at a secondary level hospital in Johannesburg, South Africa studied a cohort of 321 vertically exposed children. The diagnostic accuracy of 2 oral fluid HIV tests, OraQuick and Orasure was evaluated in this study. A serum and 2 oral fluid HIV tests were performed at 12 months of age in a cohort of perinatally exposed infants during a 14-month period proceeding October 2003. The 3 HIV tests were performed independently of each other by personnel blinded to the child's true HIV infection status, the reference standard used for comparison. The true HIV infection status of 97% children was determined. In comparison with serum HIV testing results, oral fluid HIV tests reduced the percentage of children requiring repeat HIV testing from 45% to 8-12%. OraQuick reported a sensitivity and specificity of 87% and 97% respectively. The ability of oral fluid and serum to predict an HIV uninfected status were comparable with negative predictive values >99%. The study concluded that oral fluid perform well in children and have the potential to increase accessibility and acceptability of HIV diagnosis for infants in poorly resourced prevention of mother to child transmission (PMTCT) programs.[28]

In another study, in South Africa, the affordability of early HIV testing of infants in poorly resourced PMTCT programme was assessed. It was concluded that additional marginal investment by government to access an earlier HIV diagnosis for infants could triple the efficacy of PMTCT programmes in identifying HIV-infected children for medical management and improved quality and quantity of life. Early diagnosis offers

societal benefits that extend beyond economic savings.[29] The high rate of detection of HIV antibodies by the oral fluid rapid in infants underscores the need to expand access to virological assays for definitive diagnosis of HIV infection infants.

A prospective pilot study of adults, children and medical-legal cases from Gauteng, South Africa evaluated the use of whole blood and saliva for HIV antibody testing. A rapid test strip capillary flow immunoassay was used. The results were correlated with blood specimen results obtained from routine diagnostic anti-HIV assays. Whole blood specimens taken from every individual and medico-legal cases and saliva specimens taken from 76 selected cases were tested for anti-HIV antibodies. Hema-Strip HIV - 1/2, Sero-Strip HIV-1/2 and Saliva-Strip HIV-1/2 rapid test strip methodology was used. All results were correlated with the currently recommended anti-HIV assays. The whole blood test strip result correlated 100% with the traditional diagnostic results. Only two saliva test strip results tested false negative, both from marasmic and severely dehydrated babies.[30]

A comparative study of 108 adults and 64 on children to assess the specificity and sensitivity of the GACELISA test reported a specificity of 85% and 80% respectively for the adult and children groups. The sensitivity between the two groups was 97% for the adult group and 100% for the children group. It was therefore concluded that GACELISA test is useful in children since the statistical analysis revealed no difference in specificity and sensitivity between the two groups.[31]

ORAQUICK USE IN YOUTHS

Few studies have addressed the prevalence of HIV among youths in developing countries. Tanzania reports that HIV among youths in secondary school students aged 15-19 contributes 3.2% of total HIV cases. The HIV prevalence among secondary school students was studied using OraQuick rapid test. The results showed 1% prevalence in the rural and 5.5% HIV prevalence in the urban area. Saliva sample collection for HIV testing was highly acceptable and was recommended by the youths for use in schools and VCT centres. [32]

PREGNANT WOMEN

The Mother Infant Rapid Intervention At Delivery (MIRIAD) study was conducted to learn more about the dynamics of mother-to-child HIV transmission and how to use the available interventions to their best advantage after offering rapid testing to women who do not know their HIV status late in pregnancy or at the time of delivery. Oraquick rapid HIV-1 antibody test was used in this study. The results were compared with EIA, and if positive confirmed with Western blot results. The women were offered antiretroviral therapy on the basis of the Oraquick results.

The MIRIAD study [33] was conducted from November 2001 to November 2003. Rapid HIV testing was offered to women at 16 hospitals in six cities who presented late in pregnancy or in labour with no recorded HIV test result. Of 5,744 women offered the rapid HIV test, 4,849 (84%) accepted. There were 38 reactive rapid tests and 34 were later confirmed to be positive for HIV. There were 4 false-positive and no false-negative rapid test results. Sensitivity of the rapid test was 100% and specificity was 99.9%. In women with reactive rapid HIV tests, intrapartum HIV therapy was started and newborns received post-exposure prophylaxis. Three infants were found to be infected with HIV, two of whom were already DNA-PCR positive at birth, indicating in utero infection. The median time from obtaining a blood sample to maternal notification of results was 66 minutes.

SURVEILLANCE STUDIES

In 2001, the validity of HIV testing using sputum from suspected tuberculosis adult patients in Botswana was investigated. This study was prompted by World Health Organization (WHO) recommendation that sentinel HIV surveillance be conducted on Tuberculosis patients. OraQuick results were compared with serum ELISA. Serum testing revealed that of the 377 patients being investigated for Pulmonary Tuberculosis (PTB), 84% were HIV positive. OraQuick assay detected HIV with 98.4% sensitivity and 98.3% specificity in gingival secretions and 97.1% sensitivity and 98.3% specificity on sputum samples.[34]

Two surveys undertaken in Botswana in the 1990s had recorded low rates of antituberculosis drug resistance, despite a three-fold rise in tuberculosis since 1989. In 2002, a third survey was undertaken to determine trends in antituberculosis drug resistance in patients with tuberculosis and to provide a nationwide estimate of HIV infection in such patients. Sputum specimens were obtained from patients nationwide in 2002, who also underwent anonymous, rapid HIV testing by use of Oraquick. Of 2200 sputum smear-positive patients and 219 previously treated patients with suspected recurrent tuberculosis, 1457 (60%) were infected with HIV. When this study was conducted the use of a rapid test for HIV screening (*Oraquick, Orasure Technologies, Bethlehem, PA, USA*) on sputum specimens in Botswana had been validated and was being used as an instrument for surveillance by national tuberculosis and HIV programmes.[35]

PATIENTS WITH VARIOUS LEVELS OF EXPOSURE TO HIGHLY ACTIVE ANTIRETROVIAL THERAPY (HAART).

To evaluate the performance of OraQuick rapid antibody test for diagnosis of HIV type 1 infection in patients with various levels of exposure to HAART, a cohort of patients whose HIV infection status was known were evaluated. The evaluation included characterizing the clinical features and antibody response characteristics associated with lower-than-expected OraQuick sensitivity. One hundred volunteers at low risk for HIV infection and 101 HIV-1 infected patients were recruited. The test demonstrated 100% specificity and 96% sensitivity. Four false-negative subjects were characterized by early initiation of effective ARV therapy and demonstrated waning serum anti-gp41 titters and Western blot band intensities.[36]

HIGH RISK POPULATIONS

The levels of awareness and use of alternative HIV tests (home collection kit, oral mucosal transudate collection kit and rapid tests) among people at high risk for HIV infection was assessed in the United States. Data was collected as part of an anonymous, cross-sectional interview study-the HIV Testing Survey (HITS)—conducted in seven states from September 2000 to February 2001. Three high-risk populations were recruited: men who have sex with men, injection drug users and high-

risk heterosexuals. Respondents were asked about their awareness and use of alternative HIV tests. The overall awareness and use of the alternative tests was limited. The most common reasons given for not using alternative HIV tests were: preference for the standard test; concern that the results could be less accurate and that alternative tests were not offered. The survey concluded that educational efforts should be evaluated to determine if promoting alternative HIV tests increases the numbers of people at risk for HIV who are tested. [37]

Several surveillance studies have demonstrated that oral fluid testing will improve access for the surveillance of: intravenous drug users; sexually transmitted disease clinics and sex industry workers. It can also be used for screening homeless persons, potential blood donors before their blood is accepted for donation, those in whom blood is difficult to collect such as the infants, haemophiliac, obese, infirm, elderly and very thin people. Physicians and dentists can also be screened using oral fluid tests.[19,34,35]

3.5 INFANT DIAGNOSIS OF HIV INFECTION

While the diagnosis of HIV in adults is relatively straightforward, establishing the HIV infection status of an infant is more complex. The HIV antibody is passively transmitted across the placenta during pregnancy and all babies born to HIV-infected women will test HIV antibody positive at birth. At a minimum, maternal antibodies continue to circulate in the infant for more than one year. After six months, levels fade and most babies who are not infected test negative for the HIV antibody by 12 months of life. Sometimes it takes HIV-uninfected children as long as 18 months to lose maternal antibodies. In contrast, infected babies continue to produce their own antibodies and their antibody test remain positive for life.[2,5,8]

Therefore, antibody HIV testing, which is the most widely available test in resource-limited nations only defines risk of infection in children under 18 months. This means, diagnosis must either wait until the baby is 18 months of age or use assays that directly detect the virus itself. These tests involve either nucleic or antigens testing .This technology is not readily available in resource limited settings, is costly and requires specialized laboratory capacity.[5]

Diagnosis is further complicated by challenges of obtaining infant specimen, obtaining adequate samples and the fact that those that continue to breastfeed continue to be at risk of acquisition of HIV infection. In the absence of early infant diagnosis, most HIV exposed infants are lost to follow up and die before the antibody testing can be performed at 15 or 18 months.[29,38,39,40]

Definitive diagnosis of HIV infection can only be made by conducting virological testing. Reactive tests performed in duplicate using one of the virological tests recommended confirms the diagnosis of HIV. If access to these virological tests is limited, WHO advises that the first virological testing be conducted at or around the first postnatal visit for the child (usually at 6-8 weeks following birth).[5] Earlier virological testing, during the first 48 hours of life of an HIV-exposed infant, can identify those infants infected in utero. Those infants infected during late pregnancy and intrapartum will have negative virological tests at that time. By the age of 4 weeks virological testing approaches 98% sensitivity.[28] A repeat test on a separate specimen should ideally be done to confirm an initial positive test. However, it is recognized that in severely

resource-constrained settings, repeat virological testing for definitive diagnosis may not be feasible or affordable.[5]

In children who were diagnosed as HIV-infected based on one positive virological test, HIV antibody testing should be performed after 18 months of age to confirm HIV infection. The precise algorithms of tests required to diagnose HIV infection for diagnostic and surveillance purposes are further explained in Figure 1.

Presumptive diagnosis of HIV infection

Presumptive diagnosis of HIV is based on the WHO guidelines. It helps to make clinical diagnosis of HIV, were virology tests are not available. Presumptive diagnosis of severe HIV disease requires HIV antibody testing of the child to confirm that the infant has been exposed to HIV. Use of a presumptive clinical diagnosis of infection in a child under the age of 18 months for initiation of ART should be accompanied by immediate efforts to confirm the HIV diagnosis with the best nationally or locally available test for age.[5,8]

Criteria for presumptive diagnosis of severe HIV disease[5,8]

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

- Infant is confirmed HIV-antibody positive
- Aged under 18 months
- Symptomatic with two or more of the following:
  - oral thrush
  - severe pneumonia
  - severe wasting/malnutrition
  - severe sepsis
- Other factors that support the diagnosis of severe HIV disease in an HIV-seropositive infants include:
  - recent HIV-related maternal death
  - advanced HIV disease in the mother
  - CD4 < 25 %.

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

3.6 CURRENT HIV TESTING PRACTISE AT THE UNIVERSITY TEACHING HOSPITAL, PAEDIATRICS DEPARTMENT

The standard HIV testing algorithm used in Zambia is the serial testing. It consists of screening with the Abbott determine rapid assay as the first line test and confirmation of the positive result with Unigold assay (Genie II rapid test was used at the time of the study). Discordant results are subjected to a third test, Bionor, which is a tie breaker. This testing algorithm has been formulated by the Zambia Voluntary Counselling and Testing (ZVCT) services based on the fact that these assays use different principles and thus when used together provide an approved HIV testing algorithm. This testing protocol has been adopted for diagnostic purposes in children above 18 months and for screening purpose in those less than 18 months old.

To scale up infant diagnosis of HIV, the Kaposis Sarcoma-Human Herpes Virus 8 (KS-HHV-8) Research and Diagnostic laboratory based in the Department of Paediatrics, University Teaching Hospital, with support from Centre for Disease Control (CDC), University of Nebraska-Lincoln and others conducted a pilot of Infant HIV diagnostic using DNA-PCR in 2006. Diagnostic testing of in-patients by DNA-PCR commenced in May 2006. The Research Laboratory is the National Infant Diagnostic Research Laboratory (NIDRL) and serves as the national centre for Infant Diagnosis of HIV. A protocol for DNA-PCR testing in HIV exposed infants has been formulated and has been adopted in the National protocol guidelines for integrated PMTCT. Two other centres in Zambia based in Lusaka and Ndola towns are conducting DNA-PCR and are also the satellite points receiving samples from other parts of the country for infant diagnosis of HIV.

CHAPTER 4

METHODOLOGY

4.1 STUDY DESIGN

The study was a cross-sectional, hospital based study conducted between December 2006 and March 2007.

4.2 STUDY SITE

The study was conducted at the University Teaching Hospital (U.T.H), Department of Paediatrics and Child Health, Lusaka, Zambia.

4.2.1 UNIVERSITY TEACHING HOSPITAL PROFILE

The U.T.H is a government tertiary hospital, having a total bed capacity of 1 863, and provides both outpatient and inpatient care. It is situated in Lusaka, the capital city of Zambia. Lusaka has a population of two million people, 50% of whom are children aged less than 15 years, Women of childbearing age constitute 20% of the population. Most patients seen at U.T.H are referred from the 29 primary health facilities run by the Lusaka District Health Management team. The U.T.H offers various facilities including 24-hour outpatient service in four clinical departments of Paediatrics, Obstetrics and Gynaecology, Surgery-casualty and Internal medicine. There are also specialized support services i.e. Dental, Ear, Nose and Throat (ENT), Chest, Urology, Oncology, Psychiatry and Occupational therapy services and a Blood Bank. Radiological services offers 24-hour services. In addition there is now a Nuclear Medicine and CT scan unit. Diagnostic services also include an ultra modern virology laboratory, which is also a WHO regional reference and quality assurance centre.

4.2.2 PAEDIATRIC DEPARTMENT PROFILE

The existing paediatric services at UTH include the Malnutrition ward (A07), Diarrheal unit (A06), Oncology unit, isolation ward (A05) and general wards (A02-A04). There is also a general and specialist outpatient clinic and the Child Health unit. The neonatal and paediatric intensive care units; emergency room (E.R-A01) with an adjacent 24-hour admission ward takes care of emergencies and intensive care services. Vaccines are available free of charge. Radiology services are also offered in the department. A satellite laboratory is attached to the Department and is able to do complete blood count (CBC), Haemoglobin levels (Hb), blood slides for malaria parasite (MPS), urine and stool examinations. More recently, CD4/CD8 counts have been introduced. The rest of the laboratory tests are done from the main hospital laboratory.

4.3 STUDY POPULATION

This study population comprised children below 18 months of age being admitted through the emergency unit (AO1) to the: admission ward; isolation ward (AO5); in-lay general wards (AO2, AO3,AO4, AO6, AO8) and the malnutrition ward(AO7) with varied medical conditions.

4.4 SELECTION OF STUDY SUBJECTS

A. INCLUSION CRITERIA

- Children 1 day to 18 months of age admitted to the Department of Paediatrics, U.T.H.
- Caregivers that were counselled and consented to having the HIV test using both oral fluid and blood.
- Consent to participate in the study.

B. EXCLUSION CRITERIA

- Children older than 18 months old.
- Caregivers that did not consent to having the HIV test.
- Refusal to consent to participate in the study.

4.5 SAMPLING PROCEDURE

A convenient sampling method was used. Children aged 1 day to18 months, whose caregiver consented to testing for HIV using oral fluid and blood and consented to take part in the study were consecutively recruited as they presented to the various wards.

4.6 SAMPLE SIZE

For cross-sectional studies, the information needed to determine the minimum required sample size is:

- An estimated of prevalence [P]
- Desired confidence level [α]
- Desired width of confidence interval [d]

Assuming one is sampling from a large population, the sample size is given by the formulae;

$N = \frac{(Z)^2 PQ}{d^2}$ where

- N= sample size
- Z = is the abscissa of the normal curve that cuts of f an area of the tail
- P = is the estimated portion of an attribute that is present in the population
- Q = 100 – P
- d = is the desired level of precision

The value of Z is found in statistical tables which contain the area under the curve. It is a fixed value 1.96.

P, the estimated sensitivity and specificity in this study was 95%.

Q was 100 -95 = 5

The desired precision, d, was ± 3%

The desired confidence level was 95%

The resulting sample size is demonstrated below:

$$N = \frac{[1.96 \times 1.96] \times [95 \times 5]}{[3 \times 3]} = \frac{3.8416 \times 475}{9} = 1\,824.76/9 = 202.75\ (203)$$

Each arm of patients, (the negative and positive HIV patients) had to comprise 203, bringing the total sample size to a minimum of 406. This study recruited over 100% of the estimated minimum sample size. One thousand patients were recruited.

4.7 DATA COLLECTION

Written consent was obtained from the caregivers to participate in the study. The data collection and management was supervised by the Principal Investigator. The data collection instruments were a structured interviewer administered questionnaire and log books. Once recruited into the study, information was collected from the caregiver in a face to face interview by a nurse-counsellor using a standard questionnaire. The information collected included social demographic data, caregiver and patient details, clinical data and the laboratory results of the OraQuick, Abbott Determinant, Genie11 and/or DNA-PCR. Log books and registers were prepared before commencement of the pilot and it was the responsibility of the testing individuals to document all sessions.

4.7.1 Training

All counsellors and nurses who participated in performing the OraQuick test were trained to use the test kit before the study began. The one day training took place in October 2006. The components of the training included a theory session giving information on OraQuick i.e. biological principles of test, performance characteristics, quality control and a practical session on how the test is performed. A counselling session was also conducted to encompass information on OraQuick and DNA-PCR tests.

4.7.2 Preparations of the test device

Storage temperature requirements of 2-17 ^{0} Celsius were met. Test devices were stored unopened in the KS/HHV-8 Diagnostic and Research laboratory and temperatures were checked and documented daily. Test controls were stored in the refrigerator and used only when they had attained room temperature.

4.7.3 Preparation of the test area

All the material had to be available before testing began. The testing area had adequate natural or artificial lighting, and thermometers were in place to ensure temperature ranges were maintained with in the range 15 ^{0}-27 ^{0} C. Appropriate space and a flat surface were available at each test site with hand washing facilities in close proximity to the room and hand sanitizers were used.

4.7.4 Quality Control

4.7.4.1 Internal controls

Each OraQuick device includes an in-built control. When a complete single line develops in the triangle of the "C" location on the device, the client's specimen has been correctly loaded and travelled through the test strip, indicating a valid test. Control results were evaluated with every test. If the internal control did not produce expected results, the test was not valid and was not reported to the client, and was repeated. If a second invalid result occurred, external controls were evaluated before re-doing the test a third time.

4.7.4.2 External controls

To verify that the test device is accurately detecting HIV-1, HIV- 2 antibodies, external positives and negative controls were tested from time to time. The KS/HHV-8 Diagnostic and Research laboratory provided external whole blood controls for both positive and negative sample.

External controls were conducted under the following circumstances:

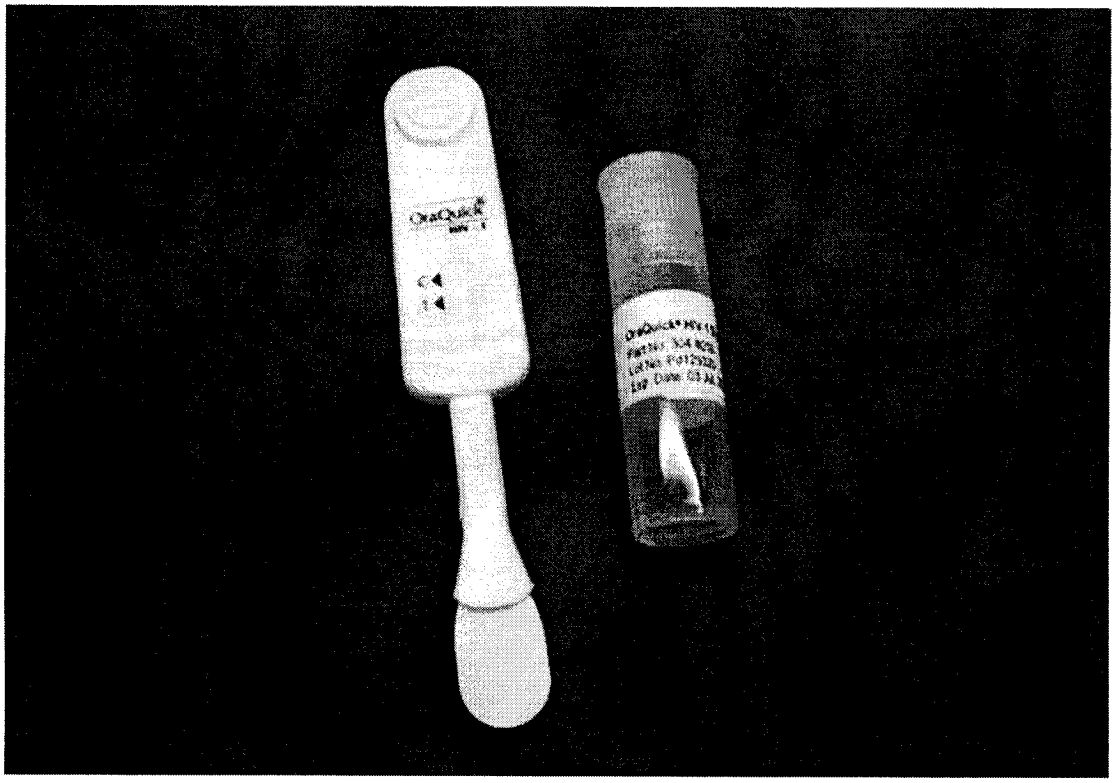
- Each new operator prior to performing testing on patients specimens
- When opening a new test kit lot
- Whenever a new shipment of test kits was received
- A second invalid test occurred
- Every day, unless more than 25 kits were run
- Temperature of the storage area fell outside of 2^0-27^0 C or
- Testing area temperature fell outside of 15^0-27^0 C

4.7.5 Testing

HIV testing was done using the OraQuick rapid HIV-1/2 Assay on oral fluid and the Abbott Determine HIV-1/2 Rapid Antibody tests on whole blood conducted simultaneously as parallel HIV screening tests. The tests were performed as per manufacturers' instructions. Two positive results were confirmed with the Genie II test, as were two discordant test results. The Genie II test result was considered confirmatory and the final result for this analysis. A virological assay (DNA PCR) was also performed on whole blood specimens which had been confirmed positive with the Genie II assay. A positive DNA PCR result was considered confirmed HIV infection

whilst a negative result confirms HIV non-infection (Fig 4). The biohazard safety precautions were followed according to standards. The test results were recorded in the questionnaire, log book and on the counselling notes in the patient's file.

FIGURE 1: COMPONENTS OF ORAQUICK ADVANCED RAPID HIV-1/2 TEST KIT



4.7.6 Specimen Collection and Testing Procedures: Oral Fluid

Step 1

- Removal of the devise from its pouch.
- The flat pad should not be touched.
- An absorbent packet should be included with the devise. If no absorbent packet is present, the devise should be discarded and a new pouch obtained for testing.
- The Flat Pad should be placed above the teeth against the outer gum.
- A gently swab completely around the outer gums, both upper and lower, one time around, using the Flat Pad.
- These areas should not be swabbed; the roof of the mouth, inside of the cheek or the tongue.
- Both sides of the FLAT PAD may be used during the procedure.

Step 11

- The Flat Pad of the device should be inserted all the way into the Vial, making sure that the Flat Pad touches the bottom of the vial. The Result window on the devise should be facing towards tester.
- The timing should be set .The devise should not be removed from the vial while the test is running. Pink fluid appears and travel up the Result window. The pink fluid gradually disappears as the test develops.
- The result should be read after 20 minutes but not more than 40 minutes in a fully lighted area.
- Then the results are interpreted.

4.7.7 Interpretation of Rapid Test-OraQuick

Results were interpreted as being one of the following:

- Non-reactive (negative): One control line appears and no other lines.
- Reactive (preliminary positive): Both the control line and test line appears.
- Invalid/Indeterminate (test result can not be interpreted): Unable to determine either control and/or test line. In this situation another OraQuick test was performed on oral fluid. If the results were still indeterminate then a test on whole blood using Genie II was performed, a third rapid test was done by finger prick (Abbott Determine).

4.7.8 Post-test counselling

Only the results of Genie11 and the DNA-PCR were communicated to the caregiver, ensuring that they understood the meaning of the results. The result of OraQuick assay on oral exudates was not communicated to the patients, since the test has not yet been validated and is still being researched.

4.7.9 Documentation

Clear documentation of all results from OraQuick, Abbot Determine, Gene II and the DNA-PCR test was kept in a separate log book by the counsellors.

4.7.10 Follow up

If blood was not collected for DNA-PCR, the information was communicated to the attending physician, so that they do so before the patient was discharged.

4.8 DATA MANAGEMENT

Information collected was reviewed and counterchecked by the Principal Investigator and the Project Assistant prior to data entry. Any irregularities noted were queried and rectified before the data was entered. The data was entered by a data entry clerk, under the supervision of the data manager. Once the data was entered, consistency checks were conducted before analysis.

4.9 DATA ANALYSIS

The serologic assay, Abbott Determine was the benchmark by which the findings of the oral fluid specimens were assessed. The specificity (i.e. the proportion of children who tested negative on oral fluid specimen among those who tested negative on sera) and the sensitivity (i.e. the proportion of children who tested positive on oral fluid sample among those who tested positive on sera) of the OraQuick were estimated. The 95% confidence interval for a single proportion was considered. The level of acceptance of HIV testing using oral fluid was also assessed. Acceptability of the test refers to the proportion of people who agreed to get tested voluntarily of the eligible 1000 caregivers who were offered testing.

Appropriate statistical software (EPI-Info and SAS) were used to analyze the data. The main outcome measures were the diagnostic accuracy measures, estimated using sensitivity, specificity and predictive values. Caregiver acceptability of HIV testing method was also assessed. Concordance between test results were estimated using Kappa statistics.

CALCULATING THE ACCURACY OF HIV TESTS (WHO) [3]

Test result	Actual HIV status		
	HIV-infected	HIV-uninfected	Total
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	

A = people with HIV who test positive (**true positive**)

B = people without HIV who test positive (**false positive**)

C = people with HIV who test negative (**false negative**)

D = people without HIV who test negative (**true negative**)

A + C = all people who are truly infected with HIV

B + D = all people who are truly uninfected with HIV

- **Sensitivity**

Probability of a positive test in people infected with HIV, expressed as a percentage (A/A+C)

- **Specificity**

Probability of a negative test in people uninfected, expressed as a percentage (D/B+D)

- **Positive predictive value**

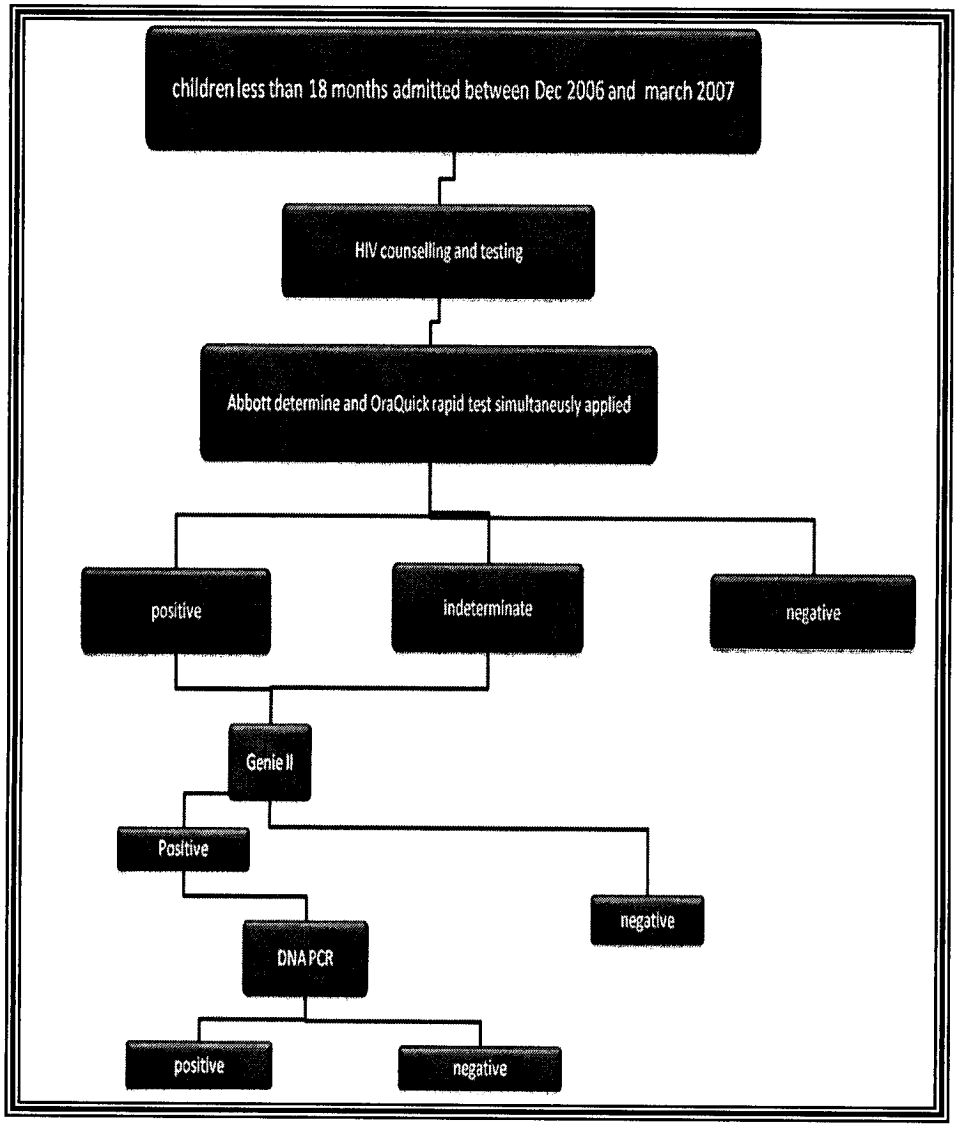
Probability that the person is HIV-infected when the test is positive, expressed as a percentage (A/A+B)

- **Negative predictive value**

Probability that the person is uninfected when the test is negative, expressed as a percentage

(D/C+D)

FIGURE 2:FLOW CHART OF PATIENT RECRUITMENT



CHAPTER 5

ETHICS

5.1 ETHICAL CONSIDERATION

Ethical approval was sought from the Research and Ethics committee of the University of Zambia prior to the initiation of the study. Permission to conduct the study in the Department of Paediatrics and Child Health was given by the Head of Department. Caregivers who expressed willingness to undergo counselling and testing were counselled and tested. The caregiver had to give written informed consent to take part in the study and to have the HIV test, using both oral fluid and blood on the child. The DNA-PCR was done on all the children with confirmed positive antibody test results, for the confirmation of their HIV status. DNA-PCR testing is currently being offered as a confirmatory test for HIV in children less than 18 months old in the Department of Paediatrics and Child Health. This test was offered to the participants in this study as part of the continuum of care being offered to all patients admitted to the Department with positive HIV sero-status.

All consent forms were written in English (the official language of Zambia) as well as in Nyanja (local language in Lusaka province).

CHAPTER 6

RESULTS

6.1 SOCIAL DEMOGRAPHIC DATA

6.1.1 PATIENT INFORMATION

The ages of the children recruited in the study ranged from 1 day to 17 months (Table 1). The median age was 2 months. Neonates comprised 38.5% of the total number of patients while the majority (45%) were children between the ages of one month to one year. More than half the patients in the study were male children comprising 53.8%. Over 95% (98.6%) of the data collected was from the biological mother. Other patients were under the care of a father, aunties and grandparents. Most of the children were in the care of a female caregiver.

TABLE 1: Socio-demographic characteristics of 1000 children

Character	Number (1000)	Percent%
Age		
< 1 month	385	38.5
1 to 12 months	448	44.8
>12 to 18months	167	16.7
Median age	2 months	
Sex		
Female	462	46.2
Male	538	53.8
Caregiver		
Mother	986	98.6
Grandparent	9	0.9
Father	3	0.3
Others	2	0.2

6.1.2 CAREGIVER INFORMATION

The age range of the parent/caregivers was between 15-25 years (Table 2). The majority (87.4%) of caregivers had been through primary and secondary level education while 9.4% had not been through any formal education.

TABLE 2: Caregiver information

Character	Description	Number	Percent%
Sex			
	Female	997	99.7
	Male	3	0.3
Age			
	15-25	530	53.0
	25-35	398	39.8
	35 & above	72	7.2
Level of education			
	none	94	9.4
	primary	435	43.5
	secondary	439	43.9
	tertiary	32	3.2

6.1.3 MEDICAL INFORMATION ON THE PATIENT

The children recruited in the study were mainly referred from private or public primary health care institutions for further management of different medical conditions (Table 3). A few patients were self-referred. The commonest cause of admission was respiratory tract infection secondary to pneumonia, accounting for 45.2% of total admissions. Septicaemia was the second commonest cause of admission and accounted for 23% of patients recruited. Diarrheal disease was the third commonest illness and comprised 13% of admissions. Other co-morbid conditions noted were malnutrition and malaria.

TABLE 3: Reasons for current admission to hospital

Medical condition	Number	Percentage%
RTI	445	44.5
Malaria	50	5
Diarrhoe	131	13.1
Malnutrition	71	7.1
Septicemia	229	22.9
Others	74	7.4

TABLE 4: Medical information on the child

Character	Description	Number	Percent
Previous Hospitalization			
	No	911	91.1
	Yes	89	8.9
Breastfeeding			
	No	171	17.1
	Yes	829	82.9
Previous HIV testing			
	No	958	95.8
	Yes	42	4.2
HIV Status			
	Negative	38	3.8
	Positive	5	0.5
	Unknown	957	95.7
ARV prophylaxis			
	N/A	796	79.6
	Not received	40	4
	received	164	16.4
Cotrimoxazole Prophylaxis			
	N/A	867	86.7
	Not received	113	11.3
	received	20	2

Approximately 90% of the patients recruited were first time admissions (Table 4). Ninety-six percent of patients had not previously been tested for HIV. Only 42 (4.2%) children had previously been tested for HIV and out of these 42, 5 were sero-positive while 38 were sero-negative for HIV (self-reporting). 204 children in this study were perinatally exposed and ideally should have received prophylaxis antiretroviral therapy but only 80% of the children did. Cotrimoxazole prophylaxis was being used in only 65% of the perinatally exposed out of the 133 that qualified to be on Pneumocystis Carinii Pneumonia (PCP) prophylaxis. Among those whom it was not applicable to be on cotrimoxazole were those that were HIV sero-positive but not yet 6 weeks old. Eighty-three percent of the children recruited were still breastfeeding.

6.1.4 MEDICAL INFORMATION ON THE MOTHER

Eighty-hundred and ninty-four (89.4%) of the mothers in this study were first tested for HIV during pregnancy. 7.2% had never been tested at all before this study. About 90% of mothers had been through the PMTCT programme during the child's pregnancy. Information on PMTCT could not be collected from 2 caregivers since the child was in the care of a relative, who was not a parent and did not have this information. The study revealed that 922 out of the 1000 caregiver knew their status, and 204/922 (22%) were positive for HIV (by verbal reporting). (Table 5)

TABLE 5: First HIV testing in mother

Time of testing	Number	Percent %
After pregnancy	6	0.6
Before pregnancy	26	2.6
During pregnancy	894	89.4
Never	72	7.2
Not known	2	0.2
Total	1000	100

TABLE 6: PMTCT information

Character		Number	Percent%
PMTCT attendance			
	No	101	10.1
	yes	897	89.7
	Not known	2	0.2
HIV status			
	Negative	722	72.2
	Positive	204	20.4
	Unknown	74	7.4
ARV prophylaxis			
	N/A	820	82
	No	15	1.5
	Not known	2	0.2
	NVP	155	15.5
	NVP+AZT	8	0.8
HAART Treatment			
	N/A	794	79.4
	no	168	16.8
	not known	2	0.2
	yes	36	3.6

Out of the two hundred and four mothers (204) who were positive for HIV, 163 received prophylactic ARV's during pregnancy and delivery, i.e. at delivery only (Nevirapine-NVP) or as a continuation from pregnancy (Zidovudine- AZT/NVP) (Table 6). Only 36 (17%) of the HIV positive mothers were receiving ARV's which were started at different time, before, during and after pregnancy.

6.2 ASSESSMENT OF ACCURACY OF ORAQUICK

RAPID HIV TEST RESULTS

TABLE 7: OraQuick, Abbott, Genie II test results

Oraquick			
		Frequency	Percent
	Negative	725	72.5
	Positive	275	27.5
Abbot			
	Negative	733	73.3
	Positive	267	26.7
Gene II			
	Negative	13	1.3
	Positive	270	27
	Not done	717	71.7

OraQuick assay detected 275 seropositive results, while the seronegative results were 725.

733 patients were seronegative and 267 positive for HIV antibodies by Abbott determine test.

Genie II, the confirmatory assay showed 270 positive results.

6.2.2 SENSITIVITY AND SPECIFICITY RESULTS

TABLE 8: OraQuick versus Abbott Determine

	Abbott Determine		Total
OraQuick Test on oral fluid	**Positive**	**Negative**	
Positive	**267**	**8**	**275**
Negative	**0**	**725**	**725**
Total	**267**	**733**	**1000**

Sensitivity (267/267 x 100) = 100% (95% CI:97.7-99.9)

Specificity (725/733 x 100) = 98.9% (95% CI:97.7-99.2)

Positive Predictive Value (267/275 x 100) = 97.1.0% (95% CI: 92.8-97.9)

Negative Predictive Value (725/725 x 100) = 100% (95% CI:98.9-100)

267 specimens were confirmed positive for HIV by Abbott determine and OraQuick. There were no false negatives reported giving OraQuick a Sensitivity and negative predictive value of 100%

Of the 733 blood specimens that were tested as non-reactive by Abbott assay, OraQuick recorded 8 of the corresponding oral fluid specimens as reactive giving a Specificity and positive predictive value of 98.9% and 97.1% respectively.

TABLE 9: OraQuick versus Abbott determine and Genie II

	Abbott determine and Genie II		
OraQuick Test on oral fluid	Positive	Negative	Total
Positive	270	5	275
Negative	0	725	725
Total	270	730	1000

Sensitivity (270/270 x 100) = 100% (95% CI: 97.3-100)

Specificity (725/730 x 100) = 99.3% (95% CI: 97.7.99.7)

Negative Predictive Value (725/725 x 100) = 100% (95% CI: 99.0-100)

Positive Predictive Value (270/275 x 100) = 98.2% (95% CI: 93.9-99.3)

When OraQuick assay was compared with the serial testing (Abbott determine as a screening test and Genie II as the confirmatory test) OraQuick was able to detect all the 270 confirmed positive results obtained by Genie II, thus the 100% Sensitivity and 100% negative predictive value. No false negatives were reported by OraQuick. Instead of 8 with the Abbott assay OraQuick reported 5 false positive out of the 730 specimens reported as negative by Abbott and Genie II, thus the specificity of 99.3%. The specificity of OraQuick was 100% with either Abott only or in combination with Genie II. The positive predictive value improved from 97.1% with Abbott alone to 98.2% when Abbott is combined with Genie II.

TABLE 10: Summary showing performance of OraQuick against Abbott/Genie II

Tests	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Kappa statistic	P-value
Oraquick vs. Abbott	100 (97.7-99.9)	98.9 (97.2-99.2)	97.1 (92.8-97.9)	100 (98.9-100.0)	0.967	<0.001
Oraquick vs. Abbott determne and Gene II	100 (97.3-100.0)	99.3 (97.7-99.7)	98.2 (93.9-99.3)	100 (99.0-100.0)	0.984	<0.001

6.3 ACCEPTABILITY OF ORAL FLUID HIV TESTING

TABLE 11: Preferences of mode of HIV testing

Testing preference before testing				
		Frequency	Percent	Cumulative percent
	Blood drawing	5	0.5	0.5
	Oral fluid	11	1.1	1.6
	Rapid needle prick	984	98.4	100
	Total	1000	100	
Preference after test				
	Blood drawing	7	0.7	0.7
	Oral fluid	807	80.7	81.4
	Rapid needle prick	186	18.6	100
	Total	1000	100	

The preferences of modes of testing were quite variable. Patients were asked their preference of testing during the interview before the child was tested and after the child was tested. The choices were blood drawing from the femoral vessels, finger prick on the index finger and oral fluid by swabbing the mouth. 98.4% preferred blood collection by finger prick initially but after testing, 80.7% preferred testing by oral fluid. The choice of oral fluid testing improved from 1.1% before the test to 80.7% after the testing experience.

CHAPTER 7

DISCUSSION

This study was conducted between December 2006 and March 2007. A total of 2,278 children were admitted to the Department of Paediatrics and Child Health, U.T.H, Zambia during this period. The Paediatric Centre of Excellence (PCOE), U.T.H. quarterly reports have shown that at any time 60% of Paediatric in-patients are within the age group less than 18 months. Sixty-seven (67%) of the admissions during the study period were children within this age group. All the patients admitted during this period were counselled to be tested for HIV and 99% acceptance rate was reported. Of the 1,531 children below 18 months of age tested for HIV, a total of 1,000 patients were recruited for this study. This translated into an overall participation rate of 65.3% (1000/1531).

The high levels of acceptance of HIV testing have prompted the introduction of "Provider Initiated Counselling and Testing". This is an initiative were health care providers are being encouraged to offer the HIV test to all patients they come into contact with.[42]

7.1 SOCIAL DEMOGRAPHIC INFORMATION

The age ranges of the patients in the study were 1 day to 17 months. There was an almost equal distribution between male(53.8%) and female(46.2%) patients. Ninety-nine (99%) of patients were in the care of a female caregiver, 98.6% of whom were the biological mothers of the patient. The caregivers were mostly (53%) within the age group 15-35 years old. Majority of the caregivers (91% [906/1000]) had attained some formal education at various levels from primary to tertiary education.

7.1.1 MEDICAL INFORMATION ON THE MOTHER AND CHILD

The patients in this study were referred from different primary health centres and private hospitals within Lusaka for the management of various medical conditions. The commonest reason for referral and admissions was respiratory tract infections (45.2%). Pneumonia was the secondary cause of the respiratory infections. A study by Professor Chintu et al at the University Teaching Hospital in Zambia looking at the impact of HIV on common paediatric illness, found Pneumonias to be the commonest cause of hospital admission. This trend was reported in both the HIV seropositive and seronegative children. It accounted for 28% of the total admissions.[41] A similar study looking at the impact of HIV-1 infection in South Africa reported respiratory conditions to be the commonest condition at admission and acute pneumonias as the commonest primary diagnosis[42] The second commonest cause of admission was Septicaemia, which accounted for 23% of the total admissions. Of the children recruited 7.1% had severe malnutrition. The co-morbidities in malnourished children were diarrheal disease and respiratory tract infections.

Mothers, as caregivers were specifically recruited to improve accuracy of information collected on the child and mother. It was noted that 90% of mothers had been through PMTCT during antenatal clinic attendance whilst they were expecting the child under study. Eighty-nine percent were first tested for HIV during the antenatal period through the PMTCT programme. Mother to child transmission of HIV accounts for 90% of paediatric HIV.[8] Reported cases of mother-to-child-transmission range from 25%-40% in some African countries.[45] In Zambia the overall HIV transmission rate without PMTCT intervention is 40%.[10]

Verbal reporting yielded an HIV prevalence of 22% among the caregivers of this study. This information may be under-reported since 30% of children were reported perinatally exposed. The Zambia sentinel surveillance system shows an HIV prevalence of 6.7% in rural areas to 31.8%in urban areas.[10] Lusaka town has the highest HIV prevalence in the country of 20% among the adults.[43] However, despite the high prevalence of HIV, and despite the majority knowing their status, very few caregivers had their children tested for HIV prior to the child's current admission to hospital. Only 43 out of 1,000 caregivers had their children tested and knew their children's HIV status.

7.2 ASSESSMENT OF ACCURACY OF ORAQUICK

Comparison of Accuracy of OraQuick with Abbott determine

In this study, evaluation of OraQuick Advanced rapid HIV 1/2, oral fluid test in children less than 18 months revealed sensitivities and specificity highly comparable to those obtained in older children and adults.[25-36] OraQuick yielded interpretable results in all the 1,000 patients recruited. OraQuick assay detected HIV in oral mucosal transudate with 100% sensitivity (267/267) [95% CI: 97.7-99.9] and 98.9% specificity (725/733) [95% CI :97.2-99.2]. The predictive values were: 100% (725/725) negative predictive value (NPV) and 97.1% (267/275) positive predictive value (PPV). Agreement between OraQuick and Abbott was high (kappa =0.97) and a significant p-value of <0.001 was obtained. According to OraSure Technologies, the OraQuick Advanced rapid HIV-1/2 antibody test had a sensitivity of 99.3% for oral fluid, 99.6% for fingerstick whole blood and 99.6% for plasma.[7] Specificity reported was 99.8% oral fluid, 100% fingerstick whole blood and 99.9% plasma. This shows that OraQuick is even more accurate when used to test blood collected by fingerprick. A surveillance following the introduction of OraQuick on the market was conducted. The aim of this survey was to test the performance of OraQuick in different states in the U.S were the test was being used. The survey concluded that the performance of the assay was as high as the manufacturers claimed.[20] Some studies conducted on OraQuick have reported high false positive results.[25] Follow up studies have, however attributed this to a cluster of tests and not to low accuracy of the test assay.[26] This study reported 8 false positives and 5 false positives OraQuick results versus Abbott and Abbott/Genie II respectively. The false positive rate reported by this study were not significant going by the high p-values and Kappa agreement reported. Accuracy of OraQuick has only been tested in children older than 12 months in one study in South African by Sherman et al.[28] The sensitivity and specificity reported was 87% and 97% respectively, with a negative predictive value of >99%. Oral fluid testing in children using various methods have reported sensitivity range of 87-100%, and specificity ranging from 80-100%.[28-31] HIV testing in various settings such as: in labour and delivery settings,[33] STI and TB clinics[27,34,35], and among patients on antiretroviral therapy [36] have also reported the high accuracy of oral fluid testing. Advantages reported in these studies are: the high level of acceptance of the test, its simplicity, non-invasiveness and it's a quick and

easy to perform test. Thus this mode of testing can be adopted in any clinical and research setting.

Comparison of Accuracy of OraQuick with Abbott determine and Genie II

When OraQuick was compared with the serial testing algorithm of using Abbott and the confirmatory test Genie II, OraQuick was more accurate in detecting HIV antibodies. Performance of OraQuick was 100% sensitive [95%CI: 97.3%-100%] and 99.3% specific [95% CI:97.7-99.7]. The PPV and NPV was 98.2% and 100%. The level of agreement between the two tests were high (Kappa=0.984). Table 11

These results demonstrate that OraQuicks' performance was higher as a confirmatory than as a screening test. Thus OraQuick can be accurately used as a confirmatory test in an HIV testing algorithm. OraQuick was recommended to be used as a confirmatory test in a study in Ethiopia. This study evaluated the use of a series of test assays to establish the VCT testing algorithm. Abbott Determine was recommended for use as a screening test and Unigold was to be used as the tie-breaker.[21] Another study in Ivory coast, at a PMTCT centre compared accuracy of Abbott determine and Genie II. Abbott yielded a sensitivity of 100% and specificity of 98,4%. The specificity and sensitivity of Genie II was 99.1% and 100% respectively.[23]

OraQuick assays in this study reported highly accurate results and can be applied in the Zambian VCT testing algorithm as screening and confirmatory tests. The test is yet to be evaluated as a tie-breaker. Western blot oral fluid test have been formulated and are in use.[19]These test however are not yet available in our clinical setting

7.3 ACCEPTABILITY OF ORAL FLUID BASED HIV TESTING

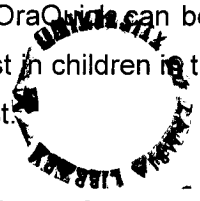
A key finding of this study was the high level of client preference for the oral fluid based HIV test. The choices of HIV testing in this study were oral fluid, needle stick/prick test on the index finger and blood drawing from a femoral or cubital fossae puncture. Clients were counselled on the choices of HIV tests available during the interview and were asked their preference before the child was tested and again after the test had been carried out by oral fluid and needle prick in some and femoral puncture in those that qualified to have a DNA PCR test done. Before testing the majority preference was by finger prick (98%) and only 1.1% found oral fluid HIV testing acceptable. But after experiencing the two or three procedures, 81% of the caregivers opted for oral fluid testing by swabbing the mouth. Oral fluid testing is a new concept in our clinical setting. Most caregivers were probably learning for the first time that oral fluid can be used to test for HIV. Thus, the low level of acceptance of testing by oral fluid initially could have been attributed to the lack of confidence in the test. Venipunture was the most unfavourable choice pre-testing and post-testing. Factors attributed to the high oral fluid HIV test preference were: less pain thus less discomfort and probably the growing cultural unacceptability of giving blood specimens especially from children in this age group, thus making oral fluid more acceptable. Acceptance of HIV testing has increased to >95% when mothers have been offered the opportunity to provide oral fluid from their children instead of blood.[17] In one study use of oral fluid reduced the percentage of children requiring repeat HIV testing from 45% to 8%. In India, oral based testing was preferred by 87% of participants for first testing and 60% for repeat testing.[27] The MIRIAD study did not demonstrate any preference for either blood or oral fluid sample collection. [33] In countries with great resistance to any blood collection, use of non-invasive saliva screening has proved an advantageous alternative. [27] In other studies acceptability of HIV testing using oral fluid ranged from 33% to 65%.[44,45,46]

CHAPTER 8

CONCLUSION

Zambia, a resource constrained country with an HIV prevalence of 16% among adults continues to have an increasing number of children who are infected with HIV. In the context of global effort to scale up paediatric HIV prevention, care, support and treatment, the government, through the Ministry of Health has introduced "Provider initiated HIV testing and counselling "as opposed to "Voluntary counselling and testing". This means that every patient admitted to the institution is offered an HIV test. "Provider Initiated Counselling And Testing" means that all health care providers should be offering patients they come into contact with HIV counselling and testing, whether they are symptomatic of HIV or not. Since the introduction of PITC, preliminary results in the Department of Paediatrics have so far indicated a high acceptance of testing of close to 85% among mothers and care givers. This in essence entails widespread HIV testing and requires a rapid, on-site, non-invasive HIV screening tool.

The main outcome measure of this study was: (a) sensitivity and specificity of the OraQuick rapid test (b) acceptance of HIV testing using Oral fluid. OraQuick rapid Advanced rapid HIV-1/2 test is as highly accurate as other blood-based ELISA rapid test. This is shown by the high sensitivity of 100% and specificity of 98.9% results obtained. Thus OraQuick can detect HIV antibodies with comparable ability to Abbott determinant assay (a screening test) and Genie II (a confirmatory test) in children less than 18 months old. This study has also demonstrated a high acceptance rate among caregivers of 81%. Thus OraQuick can be used as an alternative rapid, on-site, non-invasive HIV screening test in children in this age group. It can be used as a screening test and a confirmatory test.



According to our results, the performance of OraQuick HIV-1/2 assay is not adequate to give patients their HIV results based on a single test. There is need to validate use of OraQuick test in an algorithm involving multiple tests using the current HIV rapid tests being used. At present Unigold rapid HIV test is being used as the confirmatory test and Bionor as the tie-breaker.

CHAPTER 9

RECOMMENDATIONS

1. There is need to do further studies on all the HIV rapid tests currently being used to evaluate their performance in an algorithm and to develop an alternative rapid test based testing algorithm inclusive of OraQuick.

2. In a resource limited setting, particularly in rural health setting with limited laboratory services and skilled staff, OraQuick can be used to screen pregnant women and their children for HIV. It has proved feasible in various studies.

3. OraQuick should be made affordable in resource limited countries which need the test most.

4. There is need to sensitize the health care providers to be pro-active in providing "Provider-initiated HIV counselling and testing" to all patients they come into contact with.

CHAPTER 10

STUDY LIMITATIONS

1. If the instructions were not correctly adhered to in performing the test, there is likelihood of getting: false positives and false negatives.

2. The test kits had to be imported from another country and be transported through various weather conditions. This may have compromised the performance of the test.

3. A non-reactive result does not preclude the possibility of exposure to HIV or infection with the virus. An antibody response to recent exposure may take several weeks to reach detectable levels.

4. Negative results of OraQuick and Abbott were not subjected to Genie II, thus specificity and positive predictive values of OraQuick vs. Genie II and Abbott vs. Genie II were calculated based on assumption that the test would also produce a negative result from Genie II.

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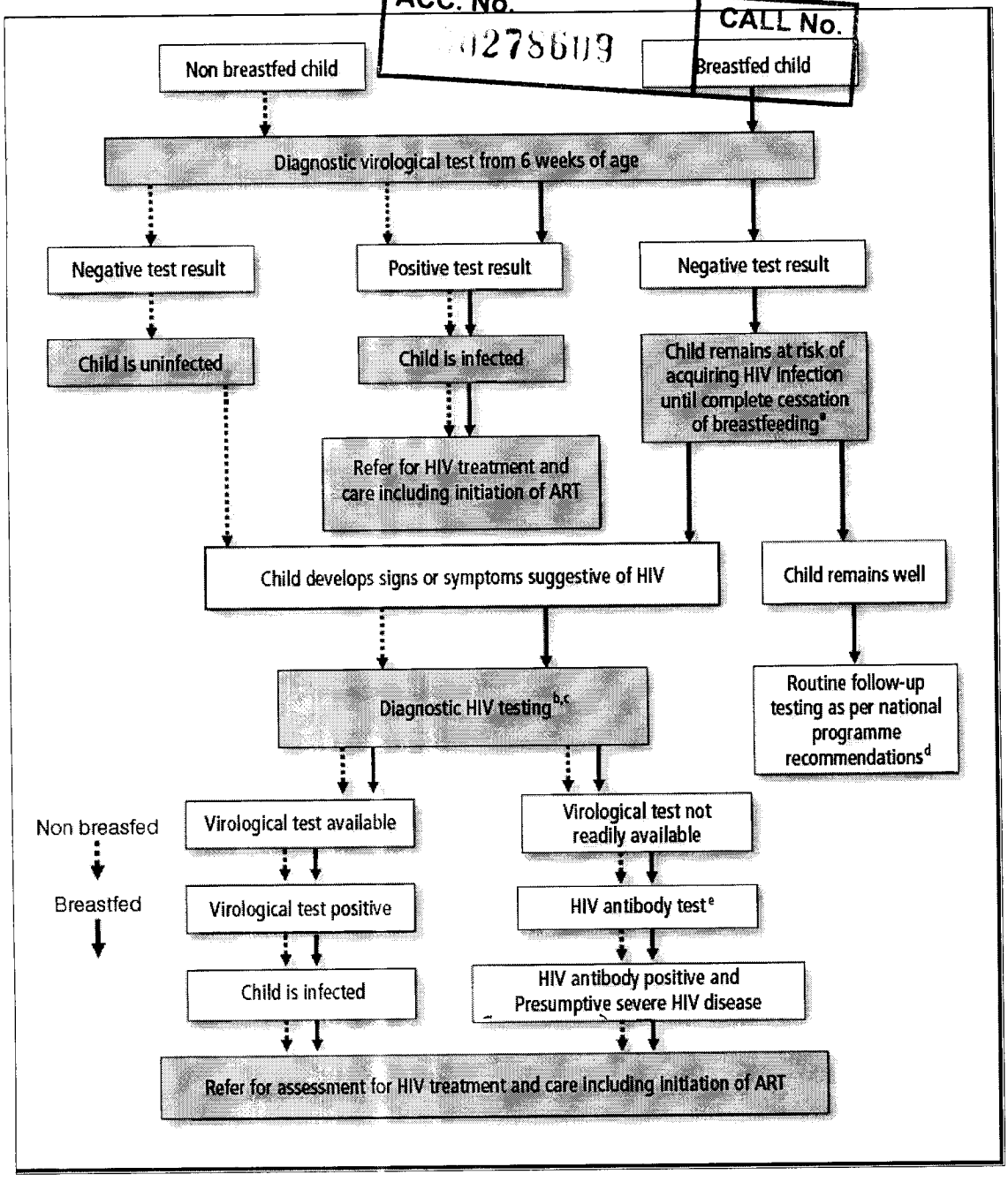
APPENDICES

CERTIFICATE OF CONSENT

I have been invited to take part in the research on use of oral fluid for testing HIV in children less than 18 months old. I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to having my child participate as a subject in this study and understand that I have the right to withdraw from the study at any time without in any way affecting the child's medical care. Equally should I consent to participate, I will be given no special services or any payments or gifts.

Name/Thumb print of caregiver	Signature/Thumb-print of caregiver/Date
Independent witness witness/Date Name/Thumb-print	Signature/Thumb-print of independent
Name of Researcher	Signature of Researcher
Date (YY/MM/DD)	

ESTABLISHING HIV INFECTION IN INFANTS AND CHILDREN LESS THAN 18 MONTHS OF AGE WITH CONFIRMED HIV EXPOSURE: (Reproduced from WHO Paediatric ART guidelines 2006)



Reproduced from the WHO Paediatrics ART guidelines for 2007

Study Number:	
File Number:	
Referral Clinic:	
Ward No:	

ORAQUICK BATCH NUMBER.....

USE OF ORAQUICK FOR HIV SCREENING IN CHILDREN LESS THAN 18 MONTHS OLD: STUDY QUESTIONAIRE

SOCIAL DEMOGRAPHIC DATA

Age Of Child *(In Months):*	**__Days__Weeks__Months**	**Sex Of Child**	☐ M ☐ F
Date of Birth of Child:	(YY/MM/DD) _____	**Age Of Parent/Caregiver**	☐ 15-25 ☐ 25-35 ☐ 35 & Above
Relationship of Caregiver to Patient:	☐ Mother ☐ Father ☐ Grandparent ☐ Uncle/Aunt ☐ Sibling ☐ Other		
Are Both Parents Alive?	☐ Yes ☐ No	**Parent(s) Deceased:**	☐ Mother ☐ Father
Sex Of Caregiver	☐ M ☐ F		
Educational Background of Caregiver	☐ None ☐ Primary ☐ Some ☐ Complete	☐ Secondary ☐ Some ☐ Complete	☐ Tertiary ☐ Some ☐ Complete

INFORMATION ON THE MOTHER

Did Mother Go Through PMTCT:	☐ Yes ☐ No		
Mothers HIV Status:	☐ Positive ☐ Negative ☐ Unknown	**When Mother was first tested for HIV:**	☐ Before Pregnancy ☐ During Pregnancy ☐ After Pregnancy ☐ N/A
Did Mother Receive Prophylactic ART's:	☐ Yes ☐ No ☐ N/A	**What medication:**	☐ NVP ☐ NVP + AZT ☐ N/A
Is Mother on HAART?	☐ Yes ☐ No ☐ N/A	**Since when:**	☐ Before Pregnancy ☐ During Pregnancy ☐ After Pregnancy ☐ N/A

MEDICAL INFORMATION ON THE CHILD

Has Child Been Tested For HIV Before?	☐ Yes ☐ No	**At What Age(months):**	_____ Months ☐ N/A
The child's Test Result For HIV:	☐ Positive ☐ Negative ☐ Unknown	**Is the Child Taking Prophylactic Cotrimoxazole?**	☐ Yes ☐ No ☐ N/A
Age when Child Started Taking Prophylactic Cotrimoxazole? (Months)	_____ Months ☐ N/A	**Did The Child Receive Prophylactic ART?**	☐ Yes ☐ No ☐ N/A
Is Child Breastfeeding?	☐ Yes ☐ No	**Age When Child Stopped Breast feeding (Months)?**	_____ Months ☐ N/A
When Other Foods Initiated (Months)	_____ Months ☐ Not yet		
Previous Hospitalization	☐ None ☐ Once ☐ Twice ☐ More Than Twice		
Reason For Previous Hospitalization	☐1. RTI-respiratory tract infection ☐2. Malaria ☐ 3.Diarrhoe disease 4. ☐ Malnutrition ☐ 5. septicaemia ☐6. Others (specify)		

Reason For Current Hospitalization	☐1. RTI-respiratory tract infection ☐2. Malaria ☐ 3.Diarrhoe disease 4. ☐ Malnutrition ☐ 5. septicaemia ☐6. Others (specify)

LABORATORY RESULTS

Abbott Determinants Test Result	☐ Positive ☐ Negative	**Oraquick Results**	☐ Positive ☐ Negative
Genie II	☐ Positive ☐ Negative		

Date when Blood Drawn For DNA PCR:	(YY/MM/DD) _____ ☐ N/A
DNA-PCR Result:	☐ Positive ☐ Negative ☐ Not Reported ☐ N/A
Preference Of Mode Of Testing (Before testing):	☐ Rapid Needle Prick Test ☐ Blood Drawing ☐ Oral Fluid
Preference Of Mode Of Testing (After testing):	☐ Rapid Needle Prick Test ☐ Blood Drawing ☐ Oral Fluid

Data Collected By: _____ Signed _____

Counter Checked By: _____ Signed _____