POINT-OF-CARE HIV TESTING FOR EARLY INFANT DIAGNOSIS DURING THE POSTPARTUM PERIOD: TIMING AND TYPE OF TEST MATTERS

Emily Rose Smith

A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology in the Gillings School of Global Public Health.

Chapel Hill
2015

Approved by:

Annelies Van Rie

Stephanie Wheeler

Michael Hudgens

Bill Miller

Jeffrey Stringer

ABSTRACT

Emily Rose Smith: Point-of-Care HIV Testing for Early Infant Diagnosis During the Postpartum Period: Timing and Type of Test Matters
(Under the direction of Annelies Van Rie)

In the past decade, large gains have been made in reducing pediatric HIV infections during the prenatal or peripartum period. However, recent changes in the World Health Organization (WHO) breastfeeding guidelines will likely shift the majority of new infant HIV infections to the postnatal period[1,2]. Although breastfeeding guidelines have changed, early infant diagnosis (EID) recommendations have not been updated.

In this dissertation, I evaluated the performance of two HIV rapid tests, Determine and Unigold, on a cohort of 121 Malawian HIV-exposed, breast-fed infants who were HIV negative at 6 weeks postpartum from 3 to 18 months of age. I also evaluated the cost-effectiveness for several EID strategies that varied in type, timing, and number of rapid tests, including an Alere test, a point-of-care virological assay, through Markov modeling.

Among 121 HIV-exposed infants, the estimated specificity increased quicker for Unigold to 100% (95% CI: 95.4, 100.0) by age 12 months compared with 95.6% (90.7, 100.0) by 15 months of age for Determine. Both tests failed to detect several incident HIV infections. Seroreversion occurred sooner with Unigold with an earlier mean time to seroreversion by 62 days (95% CI: 60, 64). Among 21 different EID strategies, the lowest costing strategy was testing once with Unigold at 9 months ($18.20 per infant) and testing twice with Alere at 6 and 15 months ($145.60 per infant). The strategies with the lowest and highest effectiveness were testing once with Unigold at 9 months (337,806 disability-adjusted-life years [DALYs]) and testing twice at 6 and 15 months with Alere (192,588 DALYs). After sequentially comparing all strategies in ranked order by costs, six remained cost-effective.

Our findings highlight that the type and timing of rapid test matters greatly in regard to accurately identifying and ruling out pediatric HIV infections. Updated guidelines for use of rapid tests in young HIV-exposed children that explicitly takes the type of test and infant age into account are urgently needed to ensure optimal care for the 1.5 million HIV-exposed infants born annually, especially in light of the new breastfeeding guidelines.

ACKNOWLEDGEMENTS

I've often thought over the past few years that the journey of going through a PhD program is made up of more than just the person pursuing the degree. Often, they are surrounded by mentors and support. I have been fortunate to have had both.

First and foremost, I want to thank my committee chair and academic advisor, Annelies Van Rie. Annelies' reputation of being brilliant and expecting excellence preceded her and I am thankful to have benefited from these qualities. She has pushed me farther than I thought I could go which, in turn, has resulted in academic maturing and becoming more of a researcher. I will always be grateful to her guidance, advice, and pursuit of excellence, as well as friendship as a woman in academia.

I would also like to thank my other committee members who in their each unique way provided mentorship and guidance. To Bill Miller, thank you for serving as a "life coach" not only concerning my dissertation but life in general. To Stephanie Wheeler, thank you for being patient in teaching me a semester's worth of modeling concepts in a few weeks. Thank you for also serving as a woman/mother academic in research. To Michael Hudgens, thank you for your guidance, patience, and availability as well as being very approachable to ask questions and learn. To Jeff Stringer, thank you for your insight regarding "in the field" applications to the questions I asked. Thank you to the entire committee for striving for a project that was not simply a way to get a degree but one that mattered in the world.

Prior to UNC, there have been several key players in my academic career, beginning with my Dad. Dad, you said the word "can't" was a bad word and I was young and naive enough to believe you were telling the truth. I am convinced that began my dreaming and ultimately led me to shoot for the stars and work hard to get there. Mrs. Dodd, Mr. Acosta, and Dr. Ross were three other influential persons, particularly in regards to nurturing a love of science. Then came Dr. Swann Adams and Dr. Alan Stevens

who both afforded the ability to stretch my academic wings, encouraged excellence, and have become lifelong friends.

I would like to thank Nancy Colvin and Valerie Hudock for their open doors and arms to all of us epidemiology students, including myself. I would also like to thank my friends I have made in the SPH, especially Katie Lesko, Jaymin Patel, Liz Cromwell, Melissa Arvay, and Kathy Lancaster. I am grateful to have made such strong professional connections and life friends.

I am also thankful for the many friends that have loved and encouraged myself and my family. Specifically, I want to extend a special thank you to the Ladies at Immanuel Baptist Church and Mark and Gayla Partin who have become extended family members.

Thank you to my parents and Mike's parents for more than I can express. I am grateful to be loved so deeply by them all without ever having to earn any of it through accomplishments. To my Dad, you have been my biggest teacher of life and learning and I'm convinced this entire journey started with you. To my Mom, you are the unsung hero with the many meals you've made, the tons of laundry you've folded, the coffee gift cards you've sent, and many other countless ways you expressed your love. To my in-loves (i.e., in-laws), I am so grateful for the support of both Mike and I through this journey. To all the grandparents and my sister, I cannot express enough how grateful I am for you all loving on Bella and Jonathan. I don't think they even knew how hard of a journey this has been because you all have loved them when I needed some help. Thank you, thank you for that.

To my kids, Bella and Jonathan. You two are my greatest joy and blessing (second to your Dad). You have reminded me what is truly important in life every day when I come home. When we started this program, you were 2 years and 3 months old. I studied while rocking at 3am by the light of an iPhone, you marked and colored on all my school books, you greeted me with cake and balloons when I finished a big test, you did your "homework" with me, we took first and last day of school pictures together (backpacks and all), and you forced me to slow down to recognize what life is all about. You have shown me grace and love and joy and provided beautiful perspective daily when you grabbed my hand and wanted to sing just one more song. A PhD is simply icing on the cake compared to being your Mom.

To Mike, we had no idea what we were about to do 4 years ago and how challenging (and wonderful) it would be. You have provided consistent strength, love, and security to us as a family. You have held us together, celebrated at each juncture, and pointed us to grace. You have provided the gracious encouragement to pursue good works and fix my eyes on faith, while also reminding me what Sabbath rest looks like. This journey is much more joyous with you. Thank you for being a safe place to call home.

Thank you to the Center for AIDS Research Network at UNC for providing financial support. Lastly, thank you to the mothers and caregivers in Malawi for participating in the study. Without you, this dissertation would not have been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
BAN	Breastfeeding, Antiretrovirals, and Nutrition Study
CDC	Centers for Disease Control
CI	Confidence interval
DT	Determine
EBF	Exclusive breastfeeding
EID	Early infant diagnosis
ELISA	Enzyme-linked immunosorbent assay
HAART	Highly active antiretroviral therapy
HEU	HIV-exposed, uninfected
HIV	Human immunodeficiency virus
Ig	Immunoglobulin
IQR	Interquartile range
MDG	Millennium Development Goal
MF	Mixed feeding
MTCT	Mother-to-child transmission
NPV	Negative predictive value
PCR	Polymerase chain reaction
PMTCT	Prevention of mother-to-child transmission
PPV	Positive predictive value
UN	Unigold
UNC	University of North Carolina
WHO	World Health Organization

CHAPTER 1: SPECIFIC AIMS

About 1.5 million HIV positive women become pregnant each year[3], resulting in a 1.5 million infants at risk of HIV infection. In 2010, the Joint United Nations Programme on HIV/AIDS Global Plan set forth ambitious goals including reducing the mother-to-child transmission (MTCT) of HIV to less than 5% by 2015. Efforts to scale up prevention of mother-to-child transmission (PMTCT) services for HIV positive mothers in low resource settings has yielded promising results, such as more widespread distribution of antiretroviral (ART) therapy and an increased uptake in PMTCT services. Despite these improvements, an estimated 260,000 children under 5 were infected with the virus in 2011[3], almost all (90%) of these infections occurred in sub-Saharan Africa.

PMTCT guidelines recommend at least 4 antenatal care visits during pregnancy, provision of ARV prophylaxis to the infant during pregnancy and delivery, and virological testing at 4-6 weeks[4]. Under these guidelines and the widespread scale-up of ART services in resource-poor settings, MTCT of HIV is likely to be successful in further reducing vertical transmission of HIV during the antenatal, delivery, and early infancy (i.e., first 6 weeks) periods. However, the current PMTCT cascade inadequately captures the postpartum period as it typically stops after delivery or, at best, after the 4-6 week infant virological test. In addition, postpartum transmission of HIV comprises 15% to 35% of new pediatric infections, suggesting current PMTCT strategies insufficiently capture the postpartum period and are not sufficient to eliminate HIV among infants[5-7]. Postpartum-acquired HIV is expected to increase given the recent changes in breastfeeding guidelines. HIV positive mothers are now encouraged to exclusively breastfeed for the first 6 months of life and continue breastfeeding for at least the first 12

months of life[8]. The extended duration of breastfeeding will increase the duration an infant's exposure, which will increase the incidence of infant HIV acquisition through breastfeeding.

Early infant diagnosis (EID) of HIV infection is critical in the survival of HIV infected children[9]. The WHO currently recommends HIV-DNA/RNA polymerase chain reaction (PCR) and ultra-sensitive p24 antigen assays for EID[10]. In countries where the risk of infant HIV infection is the highest, the utility of virologic tests are hindered by significant financial and logistical challenges. EID is also crucial in identifying seroreversion and seroconversion. Current PMTCT recommendations suggests testing infants with a rapid test 6 weeks after weaning but have not adequately addressed how to test infants in the interim period between 6 weeks of age and the cessation of breastfeeding. Rapid tests could be used in infants age 3 to 24 months, but their performance is hindered by the presence of maternal HIV antibodies. After the age of 3 months, it is unclear when rapid tests are able to distinguish between an infant HIV infection and maternal antibodies in breastfeeding populations, severely inhibiting the utility of rapid tests during the postpartum period[8,11,12 13-17]. Furthermore, different rapid tests have been shown to perform differently in infants and have not been evaluated during the entire breastfeeding period.

The Global Plan towards the elimination of new HIV infections among children by 2015 and keeping their mothers alive will only be achievable if we redefine the PMTCT cascade to encompass the mother/infant dyad throughout the entire breastfeeding period. Changes in breastfeeding guidelines will increase the number of HIV-exposed infants after the 6 week virological test. Given the importance of early ART initiation, timely, cost effective, and accurate HIV testing strategies are critical for breastfeeding infants who escaped HIV infection during the first 6 weeks of life.

To evaluate the performance of two commonly used rapid tests (Determine and Unigold) in a breastfeeding, resource-limited population and identify testing algorithms to maximize rapid test performance during the first 2 years of life, we propose the following specific aims:

Specific Aim 1:

Evaluate the performance of Determine and Unigold tests by:

Aim 1a. Determining the sensitivity, specificity, positive predictive values, and negative predictive values of Determine and Unigold rapid tests for identifying incident HIV infections, compared with the PCR reference standard, among breastfeeding, HEU infants (i.e., PCR negative at 6 weeks).

Aim 1b. Describing the Determine and Unigold rapid test results for all children with documented incident HIV infection during the breastfeeding period among infants who were PCR negative at 6 weeks.

Specific Aim 2:

Determine the median time to seroreversion in a cohort of breastfeeding, HEU infants (i.e., PCR negative at 6 weeks) during the first 3 to 18 months of life.

Specific Aim 3:

Perform a modeling study to evaluate cost-effectiveness of various infant testing algorithms to accurately identify incident HIV infection in resource poor settings.

CHAPTER 2: BACKGROUND

2.1 Dramatic improvements in PMTCT to reduce prenatal and perinatal transmission

Large gains have been made in reducing infant HIV acquisition by the effective development and implementation of prevention of mother-to-child transmission (PMTCT) programs over the past two decades and most notably after 2010. In 2010, the Global Plan set forth ambitious goals including reducing the mother-to-child transmission (MTCT) of HIV to less than 5% by 2015[18]. Efforts to scale up PMTCT services for HIV positive mothers in 22 priority countries, 1 in India and 21 in Africa, has yielded promising results, such as an increase in HIV testing and counseling among pregnant women, more widespread distribution of antiretroviral (ARV) therapy and an overall uptake in PMTCT services[19]. Rapid scale up of PMTCT services has been dramatic over the last few years with over 60% of pregnant women receiving some PMTCT service[15,20]. Coverage of ART programs has increased from 10% in 2004[21] to 62% in 2012[19]. From 2009-2011, ART prophylaxis prevented an estimated 409,000 new infant infections in resource poor settings[22]. Since 2003, new infections in children worldwide dropped by 43% and in the last 4 years alone new infections declined by 24%[22]. New HIV infections among infants have been reduced by 50% in seven of the Global Plan's priority countries[1]. Although the number of new infections among children is decreasing, the number of women newly infected with HIV has remained stable since 2009[1].

Current PMTCT guidelines

Current PMTCT guidelines recommend antenatal care visits during pregnancy, receiving HIV testing and counseling, provision of ARV prophylaxis for the HIV positive mother, and PMTCT

interventions during labor and delivery[4]. All pregnant women are encouraged to have at least 4 antenatal care visits with services for vaccinations, screening and treatment for infections, and early identification of warning signs. During the antenatal care visits, women are also offered HIV testing and counseling. If the woman is HIV positive, she is given offered an ARV regimen and encouraged to deliver in a facility equipped with PMTCT interventions. Postpartum PMTCT guidelines include following up of the infant and mother with HIV testing and linkage to HIV services if needed.

Limitations of the current PMTCT guidelines

Although PMTCT guidelines have greatly increased the number of women on ART during antenatal care, there is a paucity of guidance on how to manage HIV-exposed infants during the postpartum period for two reasons.

#1: The PMTCT cascade is mainly maternal-centric. In 2010, the WHO defined a strategic plan aimed at achieving the Millennium Development Goals (MDG) 4, 5, and 6 (MDG 4: Reduce child mortality, MDG 5: Improve maternal health, and MDG 6: Combat HIV/AIDS, malaria and other diseases) through the use of the current PMTCT structure[23]. To aid in achieving these goals, the comprehensive strategic plan included 4 components: 1) Primary prevention of HIV infection among women of childbearing age; 2) Preventing unintended pregnancies among women living with HIV; 3) Preventing HIV transmission from a woman living with HIV to her infant, and 4) Providing appropriate treatment, care and support to mothers living with HIV and their children and families. Investment needs along the PMTCT cascade projected by the Global Plan from 2011-2015 also remained largely maternal-centric with the largest increases in funding needed for more HIV testing and counseling, ARV therapy for mothers, and Option B+ availability (Figure 2)[18]. In contrast, increases in early infant diagnosis (EID) increased marginally.

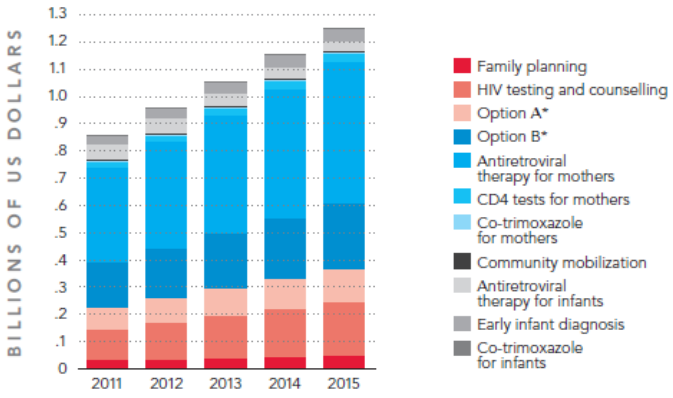


Figure 1. Investment needs in 22 priority countries from Global Plan

ARV prophylaxis is recommended for the infant during labor and delivery and extended NVP is recommended while the mother is breastfeeding. However, there remains a paucity of recommendations on how to ensure the health and well-being of the HIV-exposed infant during the postpartum period.

#2: The PMTCT cascade typically stops after delivery or, at best, after the infant's 4-6 week virological test**.** The 4-6 week virological test is vitally important as it allows for the early initiation of ART, which is linked to the survival of infected children. DNA/PCR testing remains the gold standard in diagnosing HIV infection at 4-6 weeks because of its high sensitivity and specificity. A*fter* 4-6 weeks, (Figure 3), breastfeeding children will remain exposed to HIV through for 6 to 24 months, per the updated WHO recommendations (see Section 2.3). Although the guidelines recommend further testing during this time, this recommendation is rarely implemented and many HIV positive mothers and their infants are lost during the postpartum period[21,24].

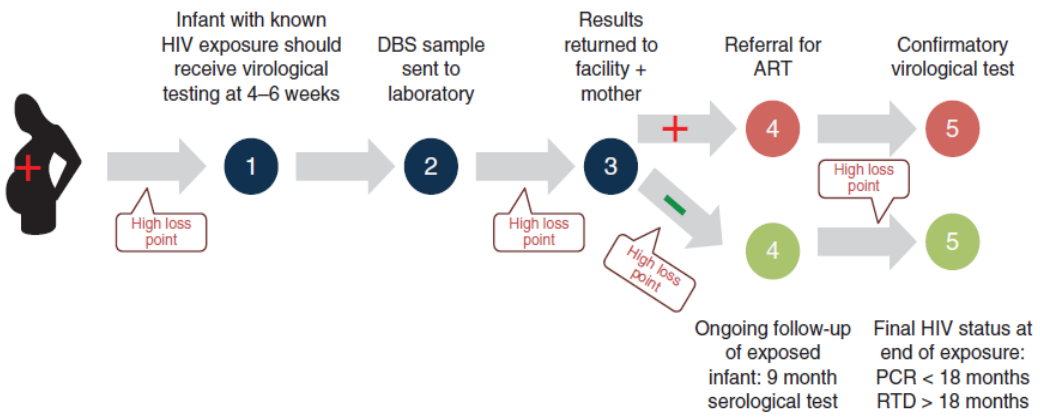


Figure 2. Early Infant Diagnosis Testing Cascade

2.2 Early infant HIV diagnosis

Accurate and timely diagnosis of HIV is crucial for the survival of HIV infected children as early initiation of treatment is required given the risk of rapid progression of disease in young infants. Without ART, up to 60% of HIV-infected infants die by the second year[25,26]. Given that EID and early ART initiation, regardless if the disease has progressed clinically or immunologically, can dramatically increase an infected infant's health outcomes, including mortality, timely detection should be a priority[9].

HIV testing methods for EID

The WHO recommends HIV-DNA or HIV-RNA PCR tests as well as ultra-sensitive p24 antigen assays for EID in resource limited settings[10]. A virological test is recommended at the first postnatal visit at 4-6 weeks and a serological test is recommended after the initial 4-6 week test if the infant develops signs or symptoms suggestive of HIV or when the infant reaches 9 months of age (Figure 3)[27]. Virological testing in HEU infants occurs again 6 weeks after discontinuation of breastfeeding to rule out HIV infection. Polymerase chain reaction (PCR)-based HIV DNA and HIV RNA assays have become the most widely used diagnostic assays for EID worldwide[10] and can detect most HIV-1 subtypes including A, B, C, D, E, G, and H. The specificity and sensitivity of HIV DNA PCR is 100% at 3 and 6 months[28-30]. HIV RNA assays have similar sensitivity and specificity values although they are not as widely used as a diagnostic tool for HEU infants.

Implementation of EID

Significant increases in EID have been seen worldwide through integrating EID with immunization services and scaling up virological testing using dried blood spots for PCR. However, these tests require samples to be transported from remote sites to centralized laboratory facilities. In developing countries where the risk of infant HIV infection is the highest, significant financial and logistical challenges hinder the utility of these tests.

EID is not only hindered by logistical factors but also greatly affected by attrition after delivery. Recent data shows that an alarming number of infants born to women with HIV do not receive testing within the first 2 months of life, with attrition numbers as high as 92%[21,24]. Recent studies in sub-Saharan Africa among lactating populations have documented attrition rates of up to 80% by 6 months, greatly impacting the utility and evaluation of virological diagnostic assays, particularly since the women have to return to the clinics a few weeks later to receive test results[31-42].

2.3 Increasing importance of MTCT during breastfeeding

Overall mortality of children with HIV before age 2 is substantially higher than children without HIV, irrespective of when they acquired the infection. However, among HIV-infected children, mortality is higher among infants who acquired the infection perinatally than among infants who acquired the infection postnatally, as summarized in a recent meta-analysis of clinical trials or cohort studies in sub-Saharan African countries[43]. Overall, the 18-month post-infection mortality risk for perinatally infected children was 60% (57%-63) while the post-infection risk for postpartum-infected children was 36% (30%-42%). Within 12 months, the differential between mortality estimates for perinatally infected or postnatally infected infants did not substantially differ. An estimated 31% of infants infected after 4 weeks had died within 12 months while 38% of infants perinatally infected died. Although the mortality associated with breastfeeding-associated infection was lower than the perinatally-infected mortality risk, we would expect both estimates to be higher in field settings. Most of the studies included in the meta-analysis offered higher standards of care with clinical follow-up that is not often representative of real-

world scenarios. Thus, it is likely the 36% breastfeeding-associated mortality risk is underestimated and highlights the need for timely and accurate detection of infection during the postpartum period.

Until recently, most of the reductions in infant HIV infections have occurred during pregnancy, delivery, and early infancy (i.e., birth to 6 week virological test)[1]. The current PMTCT guidelines of ARV interventions for pregnant and lactating women have been highly successful in reducing vertical transmission of HIV during antenatal, delivery, and early infancy (i.e., first 6 weeks) periods. As a consequence, it is estimated that over half of all new infections in high burden countries will occur during the breastfeeding period[1]. In areas with long breastfeeding, as many as 8 out of 10 newly acquired HIV infections will occur during the breastfeeding period [1]. Recent changes in the WHO breastfeeding guidelines will likely increase the duration and intensity of breastfeeding in many countries and thus increase an infant's recurrent exposure to HIV over a longer period of time, thereby increasing breastfeeding-associated MTCT.

WHO Guidelines on HIV and infant feeding (Table 1)

In 2006, HIV infected women were to choose the most appropriate infant feeding option based on their individual circumstance. Exclusive breastfeeding was recommended for the first six months of life unless replacement feeding in the local setting was acceptable, feasible, affordable, and sustainable. When such a replacement feeding was found, HIV infected mothers were encouraged to avoid all breastfeeding. When replacement feeding was not acceptable, feasible, affordable, and sustainable, HIV infected mothers should continue breastfeeding with complementary foods. Breastfeeding should then be stopped once a nutritionally adequate diet without breast milk could be provided. No recommendations regarding the use of ARV during breastfeeding were given.

Between 2006 and 2009, substantial increases in programmatic experience and research evidence provided data regarding infant feeding, infant health, and HIV. Numerous studies in several high burden countries of Botswana, India, Malawi, South Africa, and Uganda found increased infant morbidity and mortality beyond the first six months of life associated with replacement feeding among HIV-exposed infants in the absence of ARV interventions[44-52]. Infants given replacement foods suffered increased

growth deficits and other severe infections, such as bouts of diarrhea and pneumonia[44,49-53]. Similarly, exclusive breastfeeding was found to improve HIV free survival in HEU infants[54,55]. Decreased HIV transmission within the first six months of life was also found to be associated with exclusive breastfeeding compared to mixed feeding[46,55,56].

New research also highlighted that providing HIV infected mothers and/or their infants with ARV interventions could significantly reduce their risk of postnatal HIV transmission through breastfeeding. The provision of ARV along with breastfeeding through 12 months was found to prevent transmission and detrimental health benefits seen with mixed feeding[57-62]. In particular, HIV infected women who were given 3-drug antepartum ARV prophylaxis, regardless of CD4 count, through six months of study had lower rates of MTCT during breastfeeding[61]. Likewise, the Breastfeeding, Antiretrovirals and Nutrition (BAN) trial found 53% protective efficacy associated with providing 3-drug ARV prophylaxis during breastfeeding in women with CD4 > 250 cells/ul[60]. A 2011 systematic review also reported that the risk of HIV transmission during breastfeeding can be substantially reduced to 1-2% with ARV intervention[62]. These findings had profound implications for the HIV-infected mothers and their decision to breastfeed and how long to breastfeed for HIV positive mothers.

In 2010, the WHO changed the recommendations for HIV and infant feeding. HIV positive mothers are now encouraged to exclusively breastfeed for the first 6 months of life, introduce complimentary food thereafter, and continue breastfeeding for the first 12 months of life. Additionally, HIV positive mothers should receive lifelong ARV therapy or ARV prophylaxis interventions to reduce transmission during the 12 month breastfeeding period. Twelve months was chosen as the most beneficial cut-off point for breastfeeding among HIV infected mothers through modeling data since this represents the time in which breastfeeding provides the maximum survival benefit[8]. The benefit of breastfeeding until 12 months, in the presence of ARV interventions, was also found to be greater than the risk of HIV transmission. The new guidelines are recommended even in areas where ARVs are not yet available or distribution is being scaled up.

Table 1. 2006 and 2010 WHO recommendations on HIV and infant feeding.

2006 recommendations	2010 recommendations
Based on clinical stage and CD4 cell count.	Mothers known to be HIV-infected should be provided with lifelong ARV therapy or ARV prophylaxis interventions to reduce HIV transmission through breastfeeding.
The most appropriate infant feeding option for an HIV-infected mother depends on her individual circumstances. Exclusive breastfeeding is recommended for HIV-infected mothers for the first six months of life unless replacement feeding is acceptable, feasible, affordable, sustainable and safe for them and their infants before that time. All breastfeeding should stop once a nutritionally adequate and safe diet without breast milk can be provided.	Mothers known to be HIV-infected (and whose infants are HIV uninfected or of unknown HIV status) should exclusively breastfeed their infants, regardless if ARVs are available, for the first 6 months of life, introducing appropriate complementary foods thereafter, and continue breastfeeding for the first 12 months of life. Breastfeeding should then only stop once a nutritionally adequate and safe diet without breast milk can be provided.

Maternal and infant interventions for prevention of HIV transmission during breastfeeding

Treatment recommendations for HIV positive pregnant and postpartum women have been streamlined since 2010 with the adoption of a single, universal regimen of ART for life [4]. Since 2010, countries could choose between Option A, Option B, and Option B+ for pregnant, HIV positive women. Under Option A, women with CD4 counts ≤350 cells/mm receive triple ARVs starting as soon as diagnosed and infants receive a prophylactic regimen of daily nevirapine from birth through 1 week beyond the cessation of breastfeeding. Under Option B, women received triple ARVs as early as 14 weeks gestation and continued after cessation of breastfeeding and infants receive daily nevirapine or AZT from birth through age 4-6 weeks regardless of infant feeding method. Under Option B+, women receive tripe ARVs starting as soon as diagnosed and continued through life and infants receive the same prophylactic therapy as Option B. The simplified approaches of Option B and Option B+ have greatly increased the number of HIV positive women who initiated ARV while pregnant.

Even though the simplified treatment approaches of Option B and Option B+ have significantly increased the number of HIV positive, pregnant women *initiating* ART, significant challenges remain. Only 4 of the 22 priority countries of the Global Plan achieved the goal of providing ARV to 90% of pregnant women living with HIV. Among women residing in sub-Saharan Africa, only 59% received ARV therapy or prophylaxis during pregnancy and delivery in 2011[19,63]. Only 58% of mothers[64] and 29-41% of HIV-exposed infants received antiretroviral (ARV) therapy in 2009[65,66].

Problems with adherence to ART in the postpartum period

Among HIV positive women who initiated ART, high rates of non-adherence are observed in the prenatal and postpartum period (33% and 47%, respectively)[67]. Moreover, the highest rates of treatment default occur after 6 weeks postpartum and occurs progressively throughout the first 12 months[11 31,36,68,69]. Long-term ART adherence in PMTCT programs during the postpartum period has received less attention, even though the risk of nonadherence is higher[36,37,42]**.** The large drop off in treatment throughout the postpartum period results in missed opportunities to improve the health of the HIV positive mother and exposed infant through appropriate testing and treatment, if needed.

2.4 Monitoring of HIV infection during the breastfeeding period

Monitoring infant HIV infection status in the breastfeeding period is critical to timely identify HIV and to accurately rule out HIV infection. Early initiation of ART can dramatically increase the infant's likelihood of survival, particularly among perinatally-infected children. Ruling out HIV infection (i.e., identifying the time of seroreversion) is also critical to breastfeeding, HIV positive mothers of HEU infants as this gives the mother peace of mind and increases the likelihood she will continue to breastfeed.

Ideally, virological tests should be used in the breastfeeding period to accurately determine the infant's HIV infection status. As previously discussed, these tests are hindered by significant challenges in resource poor settings and repeat virologic tests throughout the breastfeeding period are not affordable in most settings. Rapid tests during the breastfeeding period could serve as a cost-effective and efficient tool for testing HIV. Repeat rapid tests are cheaper and allow for quick results that eliminate the loss-to-follow-up often seen when using virological tests that require lab transportation time, batch testing, and longer waiting time for results[11,12].

Performance of rapid tests after the 4-6 week virological test

The availability of affordable, point of care HIV testing in adult populations has revolutionized diagnosis of the HIV worldwide. Over the past several decades, numerous rapid HIV tests that detect human antibodies against HIV-specific antigens have become commercially available. In adult populations, rapid tests have demonstrated similar accuracies to enzyme linked immunosorbant assays

(ELISA) in detecting HIV infection in low resource settings, although each test performs differently in clinical practice with varied sensitivities and specificities[70,71].

Adults. Several rapid tests have been approved by the FDA for point of care HIV testing. In a study directly comparing all FDA approved rapid tests, the sensitivity and specificity of all tests exceeded 95%[72] although Unigold had the lowest sensitivity compared to Reveal G3, Multispot HIV-1/2, OraQuick Advance HIV-1/2, Stat-Pak, and Complete (96.8%, 98.9%, 99.6%, 99.5%, 98.3%, 100%, respectively)[72].

Infants. Rapid tests have been evaluated in children, but there is a *paucity of data regarding rapid tests performance among infants younger than 9 months.* Table 2 summarizes the studies which examined sensitivities and specificities of two rapid tests, Determine and Unigold, in infants younger than 18 months. All studies were conducted in resource-poor settings. Sensitivities and specificities are stratified by infant age. There were also instances where results were not stratified by infant age, making age-specific interpretation of test performances difficult. Those sensitivities and specificities are noted at the bottom of the table under "Other time points".

The estimates are limited by small sample sizes, attrition, and lack of testing among infants beyond 9 months of life[17,73-75]. Most of the studies were also conducted in non-breastfeeding populations, hindering interpretation for populations after the revised WHO breastfeeding guidelines where most women will breastfeed for longer durations.

Table 2. Sensitivity and specificity of Determine and Unigold rapid test

	Determine		Unigold	
Infant age	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
<3 months	92.6 (75.7-99.1)[75] 100.0 (NS)[73] 99.3 (98.0-99.8)[17]	8.0 (4.1-13.9) 0.0 (NS)[73]	97.0 (NS)[73]	4.0 (NS)[73]
>3-6 months	88.9 (51.8-99.7)	33.3 (22.2-46.0)		
>6-9 months	100.0 (60.7-100.0) 98.6 (?)[73] (at 7 months) 97.2 (95.5-98.3)[17] 90.0 (?)[74] (at 9 months)	82.4 (65.5-93.2) 4.0 (?)[73] (at 7 months)	31.1 (NS)[73] (at 7 months)	85.0 (NS)[73] (at 7 months)
>9-12 months	100.0 (60.7-100.0) 100.0 (NS)[73] (at 12 months)	100.0 (87.3-100.0) 55.0 (NS)[73] (at 12 months)	21.9 (NS)[73] (at 12 months)	99.0 (NS)[73] (at 12 months)
>12-18 months	90.0 (55.5-99.7)	92.0 (74.0-99.0)		
>18 months				
	Other time points			
3-18 months	98.2 (90.4-99.7)[76]	99.0 (98.4-99.4)		
1-20 months	95.3 (NS)[77]			
<18 months	95.5 (93.5-96.9)[17]	99.2 (95.6-99.9)[17]		

*NS-Not shown

Performance of alternative rapid tests

Fourth generation assays. Fourth generation assays have recently been developed and detect both HIV antibody and p24 antigen, antigen expressed in the seroconversion phase of HIV infection, providing an earlier detection of HIV infection than previous generation assays.

Adults. Field assessment of the fourth generation Determine HIV-1/2 Ag/Ab Combo rapid test was conducted among 1,009 participants in testing and counseling center in Malawi[78]. Although the test detected established HIV infection, it did not detect acute HIV infections better than the current testing algorithms of using Determine and Unigold tests. In a large study of 21,334 HIV positive persons in San Francisco, the potential for case finding of acute HIV cases was the highest for the 4th generation ARCHITECT HIV Ag/Ab Combo (99.1%; [95% CI: 91.1, 100.0]) and lower for Determine (96.6%; [95% CI: 84.7, 100.0]), Unigold (94.3%; [95% CI: 82.4, 100.0]), OraQuick Advance on blood plasma (92.8%; [95% CI: 88.2, 97.3), and Clearview Stat-Pak (92.8%; [95% CI: 83.8, 100.0])[79].

Infants. In theory, fourth generation rapid tests should be able to detect HIV infection sooner than third generation rapid tests because of early p24 antigen in infants. A recent study among infants in South Africa found that the fourth generation Determine Combo HIV-1/2 Ag/Ab Combo Test failed to detect p24Ag in 98% of HIV infected infants[80]. Even at 3 months of age, the sensitivity of the p24 antigen component was less than 10%. Among 13 infants with clinical symptoms and advanced stage disease, the test detection p24 antigen in one resulting in a sensitivity of 1.7%. The use of fourth generation rapid tests does not perform better than that of third generation HIV rapid tests, particularly among younger infants.

Oral rapid tests. Oral rapid tests serve as a less invasive option for sampling since the test requires oral fluid instead of blood. These tests are generally easier to perform and consume less time to perform.

Adults. Evaluation of the more recent OraQuick ADVANCE Rapid HIV-1/2 Antibody test also showed conflicting results. In field testing in several communities in Zambia, the test using oral fluid performed with sensitivity and specificity rates (98.7% and 99.8%, respectively) when compared with both a positive Determine and a positive Uni-Gold test[81]. Similar high sensitivity and specificity rates for the OraQuick oral rapid test have been reported in US settings[82,83]. However, another study evaluating the

performance of 4 rapid HIV tests among 21,234 HIV positive patients in San Francisco found OraQuick ADVANCE Rapid HIV-1/2 Antibody test using oral fluid was much lower (86.6%; [95% CI: 79.4-92.0]) than the other three rapid tests (OraQuick Advance using fingerstick blood, Vironostika HIV-1 Microelisa, Genetic Systems HIV ½ Plus 0)[79].

Infants. The performance of oral rapid tests among infants has not been as widely studied. A South African cross-sectional study among 597 HIV-exposed infants (birth-6 months of age) compared three oral rapid tests (OraQuick, Calypte, and Orasure)[84]. OraQuick performed best in detecting and excluding HIV exposure but the test did not detect exposure in 12% of infants. Although this study is generally relevant to the literature, it is not the goal of the proposed study since all of our infants will be HIV-exposed already. No studies have been evaluated the performance of oral rapid tests among infants who have been exposed to the disease.

2.5 The acquisition and decay of maternal antibodies

Rapid serological tests cannot distinguish between maternal antibodies and infant antibodies, making a definitive HIV diagnosis in the infant difficult[13,14]. Transfer of maternally acquired antibodies, particularly IgG antibodies, is crucial to an infant's early defense of infectious agents. Of the five antibody classes, only significant amounts of IgG are transferred across the placenta, and, after birth, IgA becomes the predominant class transferred through colostrum and breast milk. During the breastfeeding period, maternal antibodies are replaced by the child's own antibodies. This progression of the transfer and decay of maternally-acquired IgG are affected by several factors, including maternal characteristics, pregnancy outcomes, and infant age and is depicted in Figure 4.

In utero factors contributing to transplacental antibody transfer

Although the association between placental transfer of IgG and many factors, including maternal age, socioeconomic conditions, maternal nutrition, parity, and type of delivery have been evaluated, it is well established the most significant contributors affecting placentally-transferred IgG in utero are length

of gestation, placental integrity, maternal IgG concentrations.

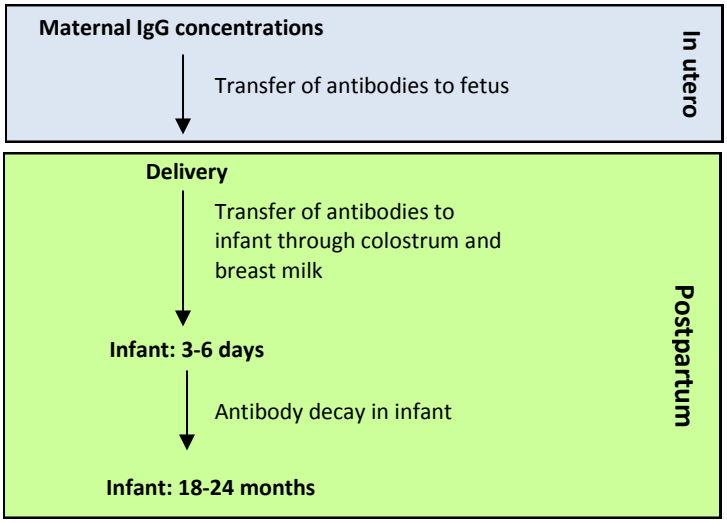


Figure 3. The acquisition and decay of maternal antibodies

Length of gestation: The most significant factor directly associated with total IgG transfer in newborns length of gestation. Maternal transfer of IgG occurs in a linear fashion beginning as early as 13 weeks, with the largest amount transferred in the last trimester[85-88]. During the second trimester, fetal IgG concentrations are only 5%-10% of maternal levels, and concentrations reach 50% of maternal levels during weeks 28-32. The largest total IgG amount acquired by the fetus occurs during the last 4 weeks of pregnancy. The association between length of gestation of IgG levels in newborns has been observed for specific antibodies against measles, mumps, diphtheria, tetanus, pertussis, influenza, rubella, and E. Coli[86,89-92].

Low birth weight: Reduced IgG transfer efficiency has been observed among low birth weight babies[87,91]. In a cohort of infants in Sri Lanka, infants with low birth weights demonstrated low levels of antibody transfer, even after adjusting for gestational age[93]. It should be noted that all mothers with low birth weight infants in the Sri Lankan study were anemic. Although birth weight may have a direct effect on IgG transfer, length of gestation serves as a more significant contributor since IgG transfer is greatly dependent on weeks in utero than the infant's weight at birth. Thus, birth weight may serve as a proxy for length of gestation.

Total maternal IgG concentrations**:** Newborn IgG levels typically correlate with maternal ones[94]. However, IgG levels can be saturated and depend on the amount of cell surface receptors present in the infant. After a saturation point, there is an inverse relationship between maternal IgG concentrations and IgG transfer to the infant, resulting in lower neonatal IgG values than maternal ones[87]. This phenomenon of lower IgG transfer is observed in certain regions in Africa where high maternal IgG values are common[87,95,96]. Specifically for HIV, mean levels of maternal IgG concentrations for all antibodies except HSV are significantly higher in HIV-infected mothers than non-infected mothers, thereby lowering the transfer of IgG to the infant[97,98].

Maternal IgG concentrations and transfer of IgG to the infant are impacted by ART exposure, both in utero and prophylactically. In a European study, infants with ART exposure in utero through maternal ART or neonatally through prophylactic therapy had lower IgG levels than infants with no ART exposure. Similarly, ART reduced the number of circulating IgG in chronically infected individuals in New York[99]. The majority of women in the European and New York studies had lower IgG levels than women in Africa, rendering it difficult to deduce if this relationship is observed in women with higher baseline maternal IgG concentrations typically seen in Africa. High baseline maternal IgG concentrations among HIV-infected women in Africa (i.e., above the saturation point) are correlated with lower IgG transfer. Thus, ART administration to HIV-infected women with high baseline IgG concentrations, as in Africa, would reduce the activation of the chronically-activated immune system, thereby *increasing* the amount of IgG transferred to the infant.

Placental integrity**:** Certain conditions, such as hypergammaglobulinaemia, malaria, and HIV, can cause pathological changes to placental tissue, thereby damaging IgG-specific Fc receptors (hRcRn) that mediate transplacental transfer to some pathogens (measles, tetanus, varicella-zoster virus, streptolysin O, pertussis, *Streptococcus pneumonia*, and HIB)[87,96,100,101]. Significant decreases in antibody transfer for pertussis, Haemophilus influenzae type B, pneumococcus, poliovirus, and tetanus have been reported in cases of maternal HIV infection or malaria in developing and developed countries[97,98,100-104]. The transfer impairment is even more pronounced among mothers with high IgG serum levels, a clinical indication of

a higher severity of disease, displaying the inverse relationship between maternal IgG levels and IgG transfer to the infants[87,97,98,105].

Clinical indicators of HIV severity, including viral load and CD4 count, are associated with placental IgG transfer. In a cohort of HIV positive, pregnant women in Nairobi, a strong inverse association between placental IgG transfer and HIV-1 viral load in the third trimester or at delivery has been observed [105,106]. In addition, women with CD4 counts less than 200 had reduced placental transfer of IgG compared with women with CD4 counts above 500. Controlling HIV viremia and boosting production of CD4 counts through effective ART at the end of a woman's pregnancy, the time where the majority of transfer of IgG occurs, could increase the efficiency of placental transfer.

Other**:** Although gestational age, maternal IgG concentrations, and placental integrity are noted most consistently in the literature as significant contributors to IgG transfer to the infant, other characteristics may also impact IgG transfer. Among women in Kenya, transfer of tetanus-specific antibodies was lower in women with low BMI, transfer of measles- and tetatnus-specific antibodies was lower in women with preterm delivery, transfer of measles-specific antibodies was lower in younger ages[101,106]. However, the majority of other studies do not find an association between BMI, maternal age, preterm delivery, and parity once gestational age, maternal IgG concentration, and co-morbidities are adjusted for[90,101].

Persistence of maternal antibodies during the postpartum period

Maternal HIV Immunoglobulin G (IgG) antibody can be detectable up to 18 months but usually become undetectable by 9 months of age. Studies from industrialized countries have reported median ages at seroreversion of 7-12 months[107-109] and an estimated 1%-2% of HEU US and European infants do not serorevert until 15 months of age[109]. In developing countries, however, there is a lack of data on seroreversion and in studies where it was evaluated the time of maternal antibody loss in infants is highly variable. Among Vietnamese HEU infants, only 22% seroreverted by 12 months[110] while 60% of South African HEU infants had seroreverted by 12 months[73]. In a recent study, the proportion of HEU South African infants who seroreverted was 20% at 4 months of age, 50% at 6 months, and 100% at 8 months of

age[17]. Maternal antibodies decline slowly throughout the first year of life in the postpartum period with a half-life of 28 to 30 days in non-breastfed infants[13,15-17]. However, there are minimal data on the persistence of maternal antibodies in breastfed populations. Of the studies describing persistence of maternal antibodies, three of the studies were in industrialized countries among non-breastfeeding populations[107-109] and three studies occurred prior to the WHO guidelines changing that encouraged breastfeeding for HIV-pregnant/lactating women and were among non-breastfeeding populations[13,15,17,73]. There were two studies which examined a mixed population of breastfeeding and non-breastfeeding HIV positive women, although the percentage of breastfeeding women was not provided[1516]. Only one study describing seroreversion documented the percentage of breastfeeding HIV positive women but this percentage was very low at 4%[110].

Postpartum factors affecting waning of placentally-acquired antibodies

After birth, IgA becomes the predominant antibody class transferred through colostrum and breast milk[111]. In addition, maternally-acquired IgG decay during the postpartum period, with the most rapid waning occurring before 9 months of age. During the first few days after delivery, colostrum is the predominant form of nutrition given to an infant and contains many protective properties, including antiviral and immunological substances. The immunoglobulin content, particularly IgG , in colostrum is much higher than that in breast milk[111]. Contributions of IgM and IgG are produced but in minuscule quantities[112-116]. Over 92%-95% of IgG is transferred in utero while the remaining 5%-8% is transferred mainly through colostrum from day 0 to day 6 and in very small quantities thereafter through breast milk[113-116]. Breast milk antibodies do not enter the infant circulation in substantial amounts and, of the five immunoglobulin classes, the most significant transfer is IgA. Although the relationship between antibody decay during the postpartum period and a myriad of maternal and infant characteristics have been examined, the most significant contributors consistently noted in the literature are infant age and maternal IgG concentrations.

Infant age**:** Infant total IgG acquired in utero is subjected to an exponential decay rate with a half-life of 35-50 days[112]. In a Swiss cohort, by 9 to 12 months of age, less than 6% of infants were

antibody positive to mumps or rubella while 9% were antibody positive to measles[117]. Similarly, other studies have found the concentration of passively-acquired IgG for measles, mumps, and rubella decreased rapidly within the first six months of life[118] and the concentration of pertussis-specific IgG decreased rapidly within the first two months of life[119].

Other than infant age, very few infant-related characteristics have been identified that impact waning of placentally-transferred antibodies. A study among rural Bangladeshi infants found measles-specific antibody concentrations decreased with age and the child's length, weight, gestational age, and parity were not associated[120]. Similarly, a Congolese study found age was the only factor influencing decay of measles antibody, even after adjusting for infant gender, birth weight, weight-for-age, maternal age, and parity[121].

Maternal IgG concentrations**.** Maternal IgG concentrations not only affect the transfer of antibodies in utero but also impact the clearance of antibodies in infants after delivery[87,94,95,120,122-124]. Elevated maternal IgG concentration reduces transplacental efficiency for the infant, resulting in lower infant IgG levels acquired in utero. Thus, the rate of infant antibody decay after delivery is faster among infants born to women with elevated IgG concentrations than infants born to women with lower IgG concentrations.

ART therapy**.** Mean time to seroreversion seems to be shorter in studies performed earlier in the HIV epidemic, particularly, before the usage of PMTCT or ART. An analysis on 3 MTCT prevention trials in Malawi found that infants born from 1989 to 1996 had a significantly shorter time to seroreversion than infants born from 2000 through 2003[16]. Similar results were noted in the US where the time to seroreversion was 4 months longer in 1990 than in 1994[125]. The most recent study evaluating differences in time to seroreversion was conducted among US infants born between 2000 to 2007[126]. The median age of antibody loss was 13.9 months among children born between 2000 to 2007 compared to 9.4, 10.3, and 10.9 months from earlier cohorts during the late 1980s and early 1990s when women did not receive ART during pregnancy[109,125,127]. Additionally, the median age of seroreversion from this study

was 3 months longer than the median ages seen in Malawi during the same time period, again indicating ART treatment could delay clearance of antibodies since ART usage was higher in the US than Malawi[16].

Although historical data, as noted above, seems to suggest maternal ART contributes to seroreversion delays, to date there have been no studies to explicitly evaluate how ART/HAART affects the time of seroreversion in infants. A small case analysis of 14 infants less than 12 months who initiated ART therapy in rural India observed that the majority of these infants did not develop sufficient antibodies for detection on rapid test due to ART[128]. However, this paper did not provide what type of rapid test was used, the number of tests performed per time period, and the ages of the children.

Other factors complicating the utility of rapid tests. Another risk factor contributing to time to seroreversion is birth weight as children with higher birth weights were more likely to serorevert at a younger age[16]. Gender, gestational age, mother's clinical stage of AIDS, breastfeeding status, and child's health history have not been found to be associated with time to seroreversion[16,109].

2.6 Current recommended HIV testing algorithms for HIV-exposed infants with a negative 4-6 week virological test

Currently, there are three main HIV testing algorithms used worldwide: 1) the 2010 WHO recommendations on the diagnosis of HIV infection in infants and children (Figure 5); 2) the CDC testing algorithm for HIV-exposed infants in settings with ART, PCR availability, and resources permit either replacement feeding or two tests per infant; and 3) the CDC testing algorithm in settings without widespread ART, limited resources for virological testing, and where most women breastfeed.

WHO Recommendations

Figure 5 describes the current WHO early infant HIV testing algorithm[27]. The group of interest given the current breastfeeding guidelines (see Section B.3), is represented in the red circle. Infants that tested negative on the 4-6 week virological test and continued to be breastfeed remain at risk for acquiring HIV until cessation of breastfeeding occurs. According to the WHO algorithm, after a negative virological test at 4-6 weeks rapid antibody testing should begin at 9 months unless signs and symptoms

suggestive of HIV occur prior to 9 months. The following two scenarios could ensue among breastfeeding infants who tested negative on a virological test at age 4 to 6 weeks:

Scenario 1: Infants who remain well are to receive an HIV antibody test beginning at 9 months of age. If the antibody test at 9 months is negative the child is assumed uninfected. A repeat antibody test is to be given six weeks after the cessation of breastfeeding and/or at 18 months of age.

Scenario 2: If infants develop signs or symptoms suggestive of HIV between 4-6 weeks and 9 months, they are to be given a virological test. If a viral test is not available, an HIV antibody test is to be performed at 9 months.

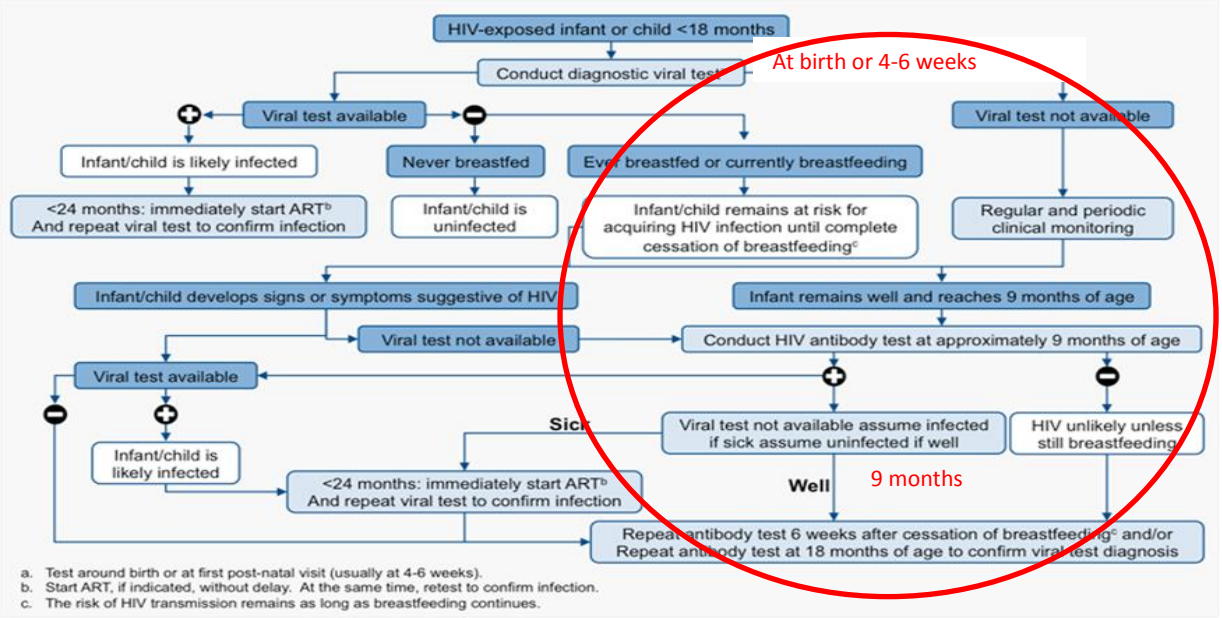


Figure 4. WHO early infant HIV testing algorithm

The testing algorithm in both scenarios recommend sparse HIV testing of HEU infants during the time between the 4-6 week virological test and 18 months of age or cessation of breastfeeding unless the infant is sick, in which it may be too late. Between age 4-6 weeks and 9 months of age, no testing is recommended unless the child is sick. This time period is approximately 7 months. A test is recommended at 9 months of age and then again 6 weeks after cessation of breastfeeding or until 18 months of age. This second risk period after age 9 months is approximately 2-9 months depending on

when the child weans. *The entire risk period from the 4-6 week virological test to the cessation of breastfeeding is 11 to 16 months*. It is not adequate enough to ensure early initiation of ART if the child does become infected through breastfeeding to simply test HIV-exposed infants a few times during the breastfeeding risk period. Another pitfall of the algorithm is that the algorithm does not describe what tests or combination of rapid tests to use at any timepoint. As previously discussed (Section B.4), these tests have widely different performances when used among infant populations in resource limited settings.

CDC testing algorithm for HIV-exposed infants in settings with ART, PCR availability, and resources permitting either replacement feeding or two tests per infant [129]

The only recommendation for an HEU infant who is younger than 9 months is to conduct PCR virological testing. If the HEU infant is 9 months of age or older, a rapid HIV antibody test is to be conducted. If the rapid test is positive, a PCR should be conducted. If the rapid test is negative and the infant has not been breastfed within the last 6 weeks, the child is considered HIV negative. If the rapid test is negative and the infant has been breastfed within the last 6 weeks, the infant is "probably" not infected but is still at risk and a repeat rapid test should be conducted 6 weeks after cessation of breastfeeding. Similar to the WHO EID testing recommendations, there is no information regarding what test to use and the frequency of and accuracy of each test. Additionally, testing occurs infrequently during the breastfeeding exposure period, placing the HEU infant at risk of late diagnosis.

CDC testing algorithm in settings without widespread ART, limited resources for virological testing, and where most women breastfeed[129]

Infants less than 6 months of age are encouraged to exclusive breastfeed. Clinical assessments are to be performed. If the child becomes ill, referral for HIV care is to occur. No mention of testing during this HIV exposure breastfeeding is given. Among children who are 6 months of age or older, a rapid HIV test is to be performed six weeks after weaning. If the antibody test is negative, the infant is considered not infected and the mother is encouraged not to breastfeed. If the antibody test is positive, the child is to continue preventive measures until definitive diagnosis can occur. If virological testing is not available, a repeat rapid test is to occur at 12-15 months of age and, if positive, definitive diagnosis is made at another repeat test at 18 months.

Similar to the WHO EID testing recommendations and the CDC testing algorithm for settings with ART, PCR availability, and resources permitting either replacement feeding or two tests per infant, there is no information regarding what test to use and the frequency for each test. Additionally, testing occurs even more infrequently than the other testing algorithms during the breastfeeding exposure period, placing the HEU infant at risk of late diagnosis. Given increasing access to virologic testing for EID, the utility of this algorithm is outdated for most settings. Nonetheless, testing recommendations are briefly outlined.

2.7 Significance of the proposed project: The intersection of the current PMTCT structure, current EID testing algorithms, and 2010 WHO breastfeeding recommendations

Current testing algorithms do not adequately describe how to optimally follow HEU infants, including what specific rapid test to use and when to administer the test, during the time between the 4-6 week virological tests through the cessation of breastfeeding. Given the effectiveness of early ARV initiation, it is unacceptable to test an infant only a few times during the high risk period of repeated exposure through breastfeeding or to wait until the child develops symptoms suggestive of HIV..

Highlighting the need for updated testing algorithms further are the recent changes in breastfeeding guidelines. The duration of breastfeeding will increase an infant's recurrent exposure to HIV over a longer period of time, which will increase the proportion of infant HIV acquired during the breastfeeding period, especially given that this risk period is not adequately captured in the current PMTCT structure. Studies evaluating rapid tests performance in resource poor settings among breastfeeding populations are especially important now that women will breastfeed for longer periods of time, increasing the risk of postnatal infection through exposure of HIV via breast milk.

The Global Plan will only be achievable if we redefine the PMTCT cascade to encompass the mother/infant dyad throughout the entire breastfeeding period. Given the importance of early ART initiation, novel testing algorithms that incorporate this risk period and are acceptable in resource limited settings are more critical now than ever among those that escaped HIV infection during the first 6 weeks of life.

CHAPTER 3: DESCRIPTION OF THE STUDY POPULATION AND DATA COLLECTION

3.1 Study setting

The data used for this study came from a community-based cohort study examining the effects of HIV on pediatric neurodevelopment. The study was conducted at two healthcare centers in the Blantyre region of Malawi between May 2008 and March 2012. Both clinics have PMTCT programs which provide HIV counseling and testing of all pregnant women, and offered nevirapine treatment for prevention of mother to child transmission of HIV.

In Malawi an estimated 57,000 (13%) pregnant women and 90,000 children were living with AIDS in 2008[66]. In 2010, Malawi was one of the 21 African priority countries targeted in the Global Plan to reduce MTCT of HIV to less than 5%, increase PMTCT services, and provide more widespread distribution of ARV therapy[18]. Since then, new HIV infections among infants have been reduced by 50% in seven of the Global Plan's priority countries, including Malawi[1]. Despite these significant advances, Malawi continues to have one of the highest rates of HIV positive women who did not receive effective ARV regimens. In 2012, an estimated 69%-77% of HIV positive women did not receive ARVs while 50% of women globally did not receive their ARVs[19].

In Malawi, breastfeeding practices are widespread with an estimated 94% to 95% of women initiating breastfeeding within the first hour of birth and 93% to 97% of women initiating within the first day of birth[130]. Additionally, 93% of infants are exclusively breastfed for the first month and 71% among all infants for the first six months. The median duration of exclusive breastfeeding was 4 months and 24 months for any breastfeeding. Among HIV positive women attending antenatal clinics in Blantyre and Lilongwe, Malawi, one study the estimated median duration of breastfeeding was 26 weeks and 22% of

women reported breastfeeding after 6 months[131]. In the study population, 78% of HIV positive women were breastfeeding at 6 months, 52% at 9 months, and 36% at 12 months.

3.2 Study population

Study population for the parent study

Inclusion criteria for the parent study included infants born to mothers greater than 15 years of age and included both HIV positive and HIV negative mothers. Exclusion criteria included mothers with active maternal alcohol or drug abuse or maternal chronic illness other than AIDS. All children, without congenital malformations or severe chronic illness, who were born to HIV infected mothers at the two participating health centers were screened for HIV infection at 4-6 weeks of age after appropriate HIV counseling and obtaining oral consent.

Study population for the proposed study

Of the 556 children enrolled in the parent study, 96 perinatally infected infants, 170 children were born to HIV negative mothers, and 290 children born to HIV positive mothers. Children enrolled in the proposed study were eligible for inclusion if they were born to an HIV infected mother, had a negative PCR test at 4 to 6 weeks of age, and had a Determine or Unigold rapid test performed after 4 to 6 weeks of age. Rapid testing in the parent study began 9 months after the start of the study and children without any rapid testing before this 9 month period were excluded. Children were also excluded if they had a rapid test performed but the type of rapid test (Determine or Unigold) was not collected. The final dataset for the proposed study includes 121 children born to an HIV positive mother and were deemed HIV negative at 4 to 6 weeks by PCR.

3.3 Data Collection for the parent study

Procedures

Data was collected at two health centers in Blantyre, Malawi. Procedures for HIV-exposed infants from study enrollment and throughout the breastfeeding period are outlined in Table 3. HIV DNA PCR testing using version 1.5 of the Amplicor HIV-1 DNA test kit (Roche, Basel, Switzerland) was performed at 4-6 weeks or closest to that time to determine infant HIV infection. Confirmation of a

positive PCR result was sought through a second PCR or through HIV RNA viral load testing. Children enrolled in the study were asked to visit the clinic at 10 weeks and every 3 months between age 14 weeks and 18 months, and every 6 months between age 18 and 42 months. Blood was collected at all time points, unless HIV seroconversion was documented at one of these time point. At ages 14 weeks, 6, 9, 12 and 15 months blood was collected for storage for biomarkers and HIV DNA PCR testing. Blood was taken for hemoglobin at each visit. HIV rapid tests using Unigold® and Determine® test kits were performed at 6, 9, 12, 15, and 18 months.

Table 3. Procedures for HIV-exposed infants

	Age 6 weeks Routine vaccination visit	**Age 10 weeks Routine vaccination visit**	**Age 14 weeks, 6, 9, 12 & 15 months**	**Age 18, 24, 30 months**	**Age 36 and 42 months**
	Care	**First Study Visit**	**Follow up study visits**		**Follow-up visits**
Oral parental permission for HIV test of child	√			√	
HIV DNA PCR testing	√				
Post test Counseling		√			
Obtain parental permission for study participation		√			
Socio-economic & demographic questionnaire		√	√	√	√
Clinical history		√	√	√	√
Clinical exam		√	√	√	√
Neurological exam		√	√	√	√
Bayley Scales of Infant Development III		√	√	√	√
Hemoglobin		√	√	√	

Data collection procedures for the mother or primary caregiver, if no biological mother, are outlined in Table 4. Maternal or primary caregiver clinical history and sociodemographic information was collected at enrollment and updated at each follow-up visit. Sociodemographic data collected included marital status, level of education, employment status, caregiving responsibilities, members living in the household, household characteristics (i.e., toilet facilities, availability of electricity, building materials of the house and roof), assets and finances, utility of a mobile phone, and health of the child's father. Clinical history included ARV regimen, usage of nevirapine at birth (maternal and infant), co-morbidity

assessment, disruptions in work due to illness, and hospitalizations. Changes in maternal/primary caregiver contact information, primary caregiver status and clinical history were updated throughout the follow-up period. Missed visit date and attempts to contact the primary caregiver were recorded as needed. Unscheduled visit dates, reason for the unscheduled visit, and diagnoses at the unscheduled visit were recorded as needed. Selected infant blood samples collected in the parent study were shipped from Malawi to the University of North Carolina at Chapel Hill (UNC).

Table 4. Procedures for HIV positive mothers or primary caregivers

Form	Description	Age 10 weeks (Enrollment)	Follow up Visits	Unscheduled visits	Used as needed
Patient Contact Information Form	Used to record phone number, address and tracing permission	√	Update		
Study Summary Sheet	Used to track dates of lab procedures and changes in HIV status	√	Update	Update	
Immunization Record Form	Used to record immunization history of the child	√	Update		
Maternal Clinical History and Socio-demographics OR Primary Caregiver Clinical History and Socio-demographics Form	Records information on the child's home environment, health of the caregiver and parents.	√			
Clinical Form	Records clinical history of the child, clinical examination, neurological examination and AIDS staging	√	√		
Primary Caregiver Clinical History and Socio-demographics follow-up	Updates information on the caregiver's health and the child's home environment		√		
Change in Primary Caregiver	Used to record changes in primary caregiver if there is a change				√
Missed Visit Form	Used to record information when a child misses a visit and attempts to contact the family and reschedule.				√
Unscheduled Visit Form	Used to record information about an unscheduled visit to the study clinic			√	
ARV treatment Log	Log to record treatment with ARVs				√
Inactivation of Study Participation Form	Used if a child discontinues the study or dies before the end of the study				√

Rapid test results for the parent study

There were 121 children that were followed from their 4-6 week negative PCR test. Table 5 describes the rapid testing that was completed on the 121 children from 2 months through 24 months. According, these children would return to the clinic for rapid testing every 3 months until 18 months of

age and then again at age 24 months. However, there were many times when the children were brought into the clinic earlier or later than these timepoints due to various reasons, such as missed visits or coming earlier/later to a scheduled appointment.

Throughout the follow-up period for each child at each timepoint, four scenarios could have occurred:

1) Both rapid tests were run and the results agreed between the two tests (DT+/UN+, DT-/UN-),

2) Both rapid tests were run but the results were discordant (DT-/UN+, DT+/UN-),

3) Only one rapid was conducted (DT- or +/UN missing, DT missing/UN- or +), and

4) Neither rapid test was conducted (DT missing, UN missing).

Table 5 describes the number of times each scenario occurred throughout the follow-up period. In total, there were 737 Determine and Unigold tests conducted in the parent study and, of these, 392 were Determine tests and 345 were Unigold tests. There were 53 study visits in which Unigold was conducted but Determine was not and 152 study visits in which Determine was collected and Unigold was not. Missing data for these visits were a result of rapid tests being out of stock, typically resulting in only one test being conducted, or the child did not come to the appointment, resulting in both tests not being conducted. Missing data due to stock-outs totaled 53 Determine and 152 Unigold tests needing to be conducted. Missing data due to a child not coming to a study visit totaled 85 Determine and 85 Unigold tests, resulting in 138 Determine tests and 237 Unigold tests not conducted in Malawi.

Table 5. Rapid test results for parent study during follow-up period

Collected data from parent study (n=737 rapid test results)

Timepoint (month)	Results agree		Results discrepant		Determine missing, Unigold complete		Determine collected, Unigold missing	
	DT+/DT+	DT-/UN-	DT+/UN-	DT-/UN+	DT?/UN+	DT?/UN-	DT+/UN?	DT-/UN?
2	0	0	0	0	0	0	0	0
3	0	0	1	0	0	3	0	0
4	0	0	0	0	0	0	0	0
6	6	34	27	1	0	18	1	0
7	0	0	1	0	0	0	0	0
8	0	0	1	0	0	0	0	0
9	2	39	22	0	0	10	0	1
10	0	0	0	0	0	2	0	0
12	2	43	1	0	1	7	0	7
15	1	34	0	0	0	4	0	17
18	2	29	1	0	0	5	1	22
21	1	2	0	0	0	0	0	2
22	0	1	0	0	0	0	0	0
24	0	19	0	0	0	3	4	27
>24	0	18	1	0	0	3	3	18
Total DT	14	219	55	1	0	0	9	94
Total UN	14	219	55	1	1	55	0	0
Total tests	28	438	110	2	1	55	9	94

Missing data from parent study (n=152 rapid test results)

Timepoint (month)	Missing both DT and UN
	DT?/UN?
2	
3	37
4	
6	11
7	
8	
9	8
10	
12	12
15	4
18	2
21	
22	
24	2
>24	
Missing DT	76
Missing UN	76
Total missing	152

3.4 Data collection for proposed study

Per study protocol, children were scheduled to visit the study clinic at age 6, 9, 12, 15, 18, 21, and 24 months. However, data was collected when the mothers and children came for a visit, which was not always at the time of the scheduled appointment. Some children also missed scheduled visits, resulting in missing rapid test data for that timepoint. To address the issue of missing rapid test data and investigate the discordant results between Determine and Unigold results, we conducted 100 Determine and 100 Unigold rapid tests on a select group of stored samples. We prioritized samples according to the following criteria summarized in Table 6:

1. Obtain results that would provide us with the shortest seroreversion time.

Priority for testing of samples was given to earlier timepoints or timepoints that would result in a shorter seroreversion observation window. For example, if an infant was positive on both rapid tests at 3 months, did not have tests run at 6 months, and tested negative at 9 months, we wanted to obtain the test results at 6 months. The initial window to observe seroreversion would be 6 months (3 months to 9 months) while the second window with the new sample would be a shorter observation window of 3 months (3 months to 6 months). Of the 53 study visits in which Unigold was conducted but Determine was not, we obtained 14 Determine results. Of the 152 times in which Determine was collected and Unigold was not, we obtained 2 results. Of the times when both Determine and Unigold was missing, we obtained 55 Determine results and 62 Unigold results.

2. Investigate a sample of discordant rapid test results.

Of the 55 discordant results, 53 were at the 9 month timepoint or prior. We reran the discordant pairs in 33 occurrences. Of the 33 discrepant results that were retested, 27 agreed with the parent study results and were not altered in the dataset. Retesting of the remaining 8 discordant pairs resulted in different rapid tests results than the original parent study data. Results of these 8 were originally positive for Determine and negative for Unigold. After retesting, the Determine and Unigold results tested positive. Three of the 8 discrepant results had a very faint Unigold test. However, it was agreed upon by

the two laboratory managers and the other reviewer that these faint results should be considered positive, per Unigold manufacturer instructions.

3. Investigate questionable test results.

There were occasions where an infant had a 6 negative test but had a 9 month positive rapid test. This would seem like a seroconversion but was not classified as one by study investigators throughout the follow-up period. We reran these tests at 6 months and found one to be positive and one to be negative.

Table 6. Summary of UNC rapid testing

Reason for retesting	**DT**	**UN**
Missing both DT and UN results	55	62
Missing DT results	14	0
Missing UN results	0	2
Discrepancy between initial DT/UN testing	27	25
Discrepancy between initial DT/UN testing	8	9
Other questionable test results.	2	5

*DT-Determine; UN-Unigold

After this first round of retesting, we received funding from the University of North Carolina Center for AIDS Research to test all available samples needed. This resulted in a total of 492 Determine and 504 Unigold tests conducted. We compared the population with missing data versus complete data, by timepoint and by test, for characteristics including infant factors (gender, breastfeeding, nevirapine at birth) and maternal factors (maternal age, marital status, education level, and socioeconomic variables). (see Appendix A). There were no factors consistently different between the two populations. We therefore concluded the missing data population was similar to the complete case population and proceeded with the missing data population for analyses.

Table 7. Description of collected or missing rapid test data throughout follow-up, stratified by type of rapid test.

	3m		6m		9m		12m		15m		18m		Total	
Collection site	DT	UN	DT	UN	DT	UN	DT	UN	DT	UN	DT	UN	DT	UN
Malawi	1	1	66	66	63	62	52	52	56	56	57	57	295	294
UNC	75	76	31	39	29	29	35	38	20	17	7	11	197	210
Total	76	77	97	105	92	91	87	90	76	73	64	68	492	504
Total missing	45 (37%)	44 (36%)	25 (20%)	16 (13%)	29 (24%)	30 (25%)	34 (28%)	31 (26%)	45 (37%)	48 (40%)	57 (47%)	53 (44%)	234 (32%)	222 (31%)

DT: Determine, UN: Unigold

CHAPTER 4: METHODS

4.1 Specific Aim 1

4.1.1 Study Sample and Data Collection

We collected data on the performance of Unigold® and Determine® rapid tests as part of a community-based cohort study examining the effects of HIV on neurodevelopment in infants receiving care at two healthcare centers in the Blantyre region of Malawi between May 2008 and March 2012. During that period, both clinics provided HIV counseling and testing to all pregnant women and offered single dose nevirapine or zidovudine treatment to women who were HIV infected. The study included infants born to HIV-positive mothers (age ≥ 15 years) who did not have a history of alcohol or drug abuse or chronic illness other than AIDS. For the analysis presented here, only children who were negative by HIV DNA PCR at the time of enrollment were included.

HIV DNA PCR testing using version 1.5 of the Amplicor HIV-1 DNA test kit (Roche, Basel, Switzerland) was performed at enrollment, which took place at approximately 6 weeks of age (median age 6.4 weeks, interquartile range [IQR] 4.3-15.1 weeks). Children were scheduled to visit the study clinic at age 10 weeks and every 3 months between age 14 weeks and 18 months. At all timepoints, clinical and sociodemographic information were collected, a fingerprick was performed for hemoglobin testing, dried blood spots were collected for storage for HIV DNA PCR assays, and a venous whole blood sample was taken for storage at ≤ -70 degrees Celsius. Confirmation of infant HIV infection detected by a positive HIV DNA PCR result was done through a second HIV DNA PCR assay or a HIV RNA viral load assay.

Starting in February 2009, an additional fingerprick was performed at point-of-care for Unigold® and Determine® rapid HIV tests at age 6, 9, 12, 15, and 18 months. In addition, to obtain data on rapid test performance at age 3 months, the Unigold® and Determine® rapid tests were performed on stored blood

collected at age 3 months. If data were missing at other timepoints due to failure to perform the rapid test at point-of-care or assay stock outs, rapid HIV tests were also performed on stored blood when available. Rapid testing on stored blood was conducted at a research laboratory at the University of North Carolina. Stored samples were thawed per manufacturer's instructions, and both rapid tests were performed simultaneously by a single operator. Weakly positive rapid tests results were reported as positive.

The University of Malawi College of Medicine Research and Ethics Committee and the University of North Carolina at Chapel Hill Institutional Review Board approved the study protocol. All mothers provided written informed consent and permission for participation of their infant.

4.1.2 Statistical Analysis

The 18-month cumulative incidence of infant HIV infection was estimated using an extension of the Kaplan-Meier estimator that allows for competing risks of death or loss to follow-up[132] Sensitivity and specificity were estimated in a cross-sectional manner at each timepoint (3, 6, 9, 12, 15 and 18 months), separately for Determine and Unigold. A two-week window around each timepoint was used to define a visit. Data occurring outside of the two-week window were discarded. Sensitivity for each rapid test was estimated by the proportion of infants diagnosed as HIV infected by the rapid test among all infants with confirmed infection (by PCR) at the timepoint of interest. Specificity for each rapid test was estimated by the proportion of infants diagnosed as HIV negative by the rapid test among all infants with confirmed free of HIV infection (by PCR) at the timepoint of interest.

The positive predictive value (PPV) was estimated by the proportion of infants with confirmed HIV infection among all infants who tested positive on the rapid test. The negative predictive value (NPV) was estimated by the proportion of infants free of HIV infection among all infants who tested negative on the rapid tests. PPV and NPV were determined for a range of population infant HIV incidence. To depict the levels of HIV incidence during the postpartum period currently seen throughout Africa, we used a range of 1-5% for the incidence of infant HIV infection for each of the 3-month periods. This range was selected based on data reported in two trials of antiretroviral prophylaxis in the

first two years of life among infants in Malawi, a meta-analysis to estimate incidence of postpartum HIV infection in multiple African sites, and data from the present study[133,134].

The reference standard was one positive PCR test plus a confirmatory PCR test, one positive HIV DNA PCR plus one positive HIV RNA assay before age 18 months of age, or one positive HIV rapid test at age 18 months. If a PCR test could not be performed at a specific timepoint, the result of the next available PCR result was assessed and, if negative, the infant was assigned a HIV uninfected status at the timepoint of interest. If positive, the earlier timepoint was excluded from analyses. Exact methods were used to calculate 95% confidence intervals (CIs) for sensitivity and specificity estimates. Analyses were conducted using SAS software, version 9.4 (SAS Institute).

4.2 Specific Aim 2

4.2.1 Study Sample and Data Collection

The study sample and data collection methods for Specific Aim 2 were the same as Specific Aim 1.

4.2.2 Statistical Analysis

The primary endpoint, time to seroreversion, was defined as the first occurrence of a negative HIV rapid test. Because rapid tests were performed at 3-month intervals, the exact time of seroreversion was not observed but known to have occurred in the time interval between the last positive rapid test and the first negative rapid test. The distribution of age of seroreversion was estimated using the extension of the Kaplan-Meier product limit estimator which allows for interval censored data via SAS PROC ICLIFETEST[135-137] (SAS Institute). Observations were right censored at the first occurrence of an incident HIV infection, death, loss to follow-up, or at the end of the 18 month follow-up period. We accounted for competing risks of death or HIV infection. Bootstrap sampling was used to calculate a corresponding 95% confidence interval. As each infant contributed a result for both Determine and Unigold, paired survival curves were generated. To compare the paired survival curves, the difference in mean survival times were estimated by computing the area under each curve.

4.3 Specific Aim 3

4.3.1 Analytic overview

We compared three point-of-care diagnostic assays, of which two were antibody tests (Determine and Unigold) and one was a newly developed virological assay (Alere Q HIV PCR), for several early infant testing strategies during the postpartum period. Using Markov models, we simulated each infant's trajectory from the 4 to 6 week timepoint through the lifetime.

We projected outcomes, including cost, effectiveness measured in DALYs, and overall cost-effectiveness, for a cohort of HIV-exposed infants, following a negative HIV PCR DNA test at 4 to 6 weeks postpartum, among several early infant diagnosis testing strategies in Malawi. Clinical outcomes included delayed HIV diagnoses, number of false negative, false positive, true negative, and true positive rapid tests, and infant mortality. Economic outcomes included testing costs associated with each strategy, HIV pre- and post-test counseling costs, and healthcare and treatment costs for HIV infected persons. We calculated incremental cost-effectiveness ratios (ICERs) in $/DALY: difference in testing strategies costs between two strategies divided by the difference in DALYs between the two strategies. For ICERs, all outcomes were discounted at 3%/year[138]. We examined variations in test sensitivity, specificity, loss-to-follow up rates, healthcare and treatment costs, and mortality associated with HIV infection through one-way, deterministic sensitivity analyses. We also conducted multiway sensitivity analyses, varying test performance (i.e., sensitivity and specificity), Alere costs, healthcare and treatment costs, and mortality associated with HIV infection simultaneously.

4.3.2 Early infant diagnosis algorithms

We evaluated 21 EID strategies based on a combination of three POC assays and seven timings of HIV testing (Table 8). We evaluated different timing scenarios: 1) testing at the WHO recommended time points (i.e. at 9 months and either at 6 weeks after cessation of breastfeeding or at age 18 months), 2) testing only once at 6, 9, 12 or 15 months, and 3) testing twice during the postpartum period (testing at 6 months and then either 12 or 15 months). For each timing strategy, we evaluated Determine and Unigold, two antibody assays, and Alere q HIV 1/2, a qualitative POC nucleic acid test that does not require a

laboratory or extensive training. For all strategies, all positive POC tests are to be followed up with a PCR test to confirm the HIV diagnosis.

Table 8. Description of early infant HIV diagnosis testing strategies

EID Algorithm	Testing timepoints	Point-of-care test
Current WHO recommendation	2 tests at 9 and 18 months or 6 weeks after BF cessation	1. Determine 2. Unigold 3. Alere q HIV 1/2
EID strategies with 1 testing timepoint	1 test at 6 months	4. Determine 5. Unigold 6. Alere q HIV 1/2
	1 test at 9 months	7. Determine 8. Unigold 9. Alere q HIV 1/2
	1 test at 12 months	10. Determine 11. Unigold 12. Alere q HIV 1/2
	1 test at 15 months	13. Determine 14. Unigold 15. Alere q HIV 1/2
EID strategies with 2 testing timepoints	2 tests at 6 and 12 months or 6 weeks after BF cessation	16. Determine 17. Unigold 18. Alere q HIV 1/2
	2 tests at 6 and 15 months or 6 weeks after BF cessation	19. Determine 20. Unigold 21. Alere q HIV 1/2

*BF-Breastfeeding; EID-Early infant diagnosis

WHO recommended strategies:

According to the WHO algorithm, HIV-exposed infants with a negative virological test at 4 to 6 weeks of age should be tested at 9 months and 18 months, or 6 weeks after cessation of breastfeeding, with a rapid test, unless the infant has signs and symptoms suggestive of HIV[27]. Thus, the WHO recommended strategies included 2 testing timepoints at 9 months and 18 months, or 6 weeks after cessation of breastfeeding.

Testing strategies with only one timepoint

Attrition during the postpartum period affects an overwhelming majority of PMTCT programs in resource-limited settings and even impacts randomized clinical trials where losses are closely monitored[67,139-143]. Furthermore, losses to follow-up (LTFU) are greater in the postpartum period than during the antenatal period[35,144,145]. Recent data shows that an alarming number of infants born to women

with HIV do not receive HIV testing within the first 2 months of life, with attrition numbers as high as 92%[21,24]. Likewise, other studies in sub-Saharan Africa among pregnant populations have documented attrition rates of up to 80% by 6 months[31-42]. A recent systematic review of studies evaluating LTFU along the entire PMTCT cascade found extensive heterogeneity in findings with LTFU at 3 months ranging from 4.8 to 75.2% and ranging from 50.2 to 85.1% at 12 months postpartum[146]. The overall pooled estimate at 3 months was 33.9% (95% CI: 27.6, 41.5). It is of note that many of these studies were conducted when the PMTCT programs were first being implemented and only used single dose nevirapine.

The recent introduction of the WHO Option B and Option B+ programs throughout Africa, in which all women receive antiretroviral drugs, shows encouraging retention rates of nearly 80% after 12 months on the program[147]. It should be noted that the majority of women in the program were already on ART for their own health and had significantly lower LTFU rates than women initiating ART to prevent mother-to-child transmission. Thus, the results are not entirely generalizable to all populations unless mothers are already on ART. As more countries move to the similar programs and continue to disseminate more widespread availability of PMTCT services, we expect the LTFU proportions to gradually reduce, although probably not as rapidly as in the Malawian study.

Most of the maternally-acquired HIV antibodies in HIV-exposed, uninfected infants will have seroreverted by 9 months of age[13,15-17,107-109]. In our data, 86% of infants had seroreverted according to the Determine test and 99% of infants had seroreverted according to the Unigold test by 9 months of age. The testing timepoint of 9 months was chosen as one strategy as this is the recommended vaccination time according to the WHO vaccination schedule[148]. Additionally, we evaluated testing only at 6 months or testing only at 12 months.

Testing strategies with two timepoints

Because attrition affects a large majority of PMTCT postpartum follow-up programs in many resource limited settings, we defined the worst case strategy as simply returning to the clinic at 18 months or 6 weeks after a mother ceased breastfeeding. This scenario, although not ideal, may reflect the current

reality of many PMTCT programs, especially those with the highest loss to follow-up rates. Although we do not recommend this strategy, we evaluated the cost-effectiveness to reflect the worst case scenario that could occur in HIV-prevalent countries. Furthermore, we do not anticipate this strategy to prove beneficial but we expect doing more than 1 testing timepoint will greatly improve the effectiveness of an early infant diagnosis program, thereby encouraging countries to focus efforts on at least one testing timepoint for HIV-exposed infants. Evaluating the worst case strategies allowed us to evaluate conducting at least 1 rapid test before 15 months of age as opposed to none. Furthermore, it is likely the 15 month postpartum timepoint represents 6 weeks after most women discontinue breastfeeding. The updated breastfeeding guidelines encourage women to breastfeed for 12 months and the current WHO testing guidelines advise a rapid test 6 weeks after cessation of breastfeeding. Thus, 15 months is a logical testing that represents the end of postpartum HIV risk period.

Postpartum breastfeeding-associated transmission of HIV to the infant is greatest within the first 6 weeks of life and between 6 and 12 months of age (Table 9).

Table 9. Cumulative incidence of postpartum HIV infant infection.

	Time period								
Study	**Birth**	**0- 1.5**	**1.5-3**	**3-6**	**6-9**	**9-12**	**12-15**	**15-18**	**Overall**
Our data		1.6		2.6	1.8	1	0.2	2.1	17.2
Coutsoudis, 2004		5		4.2		2.8	0.2	2.1	9.3
Taha, 2007	8.43		1.22		4.05		3.48		17.2

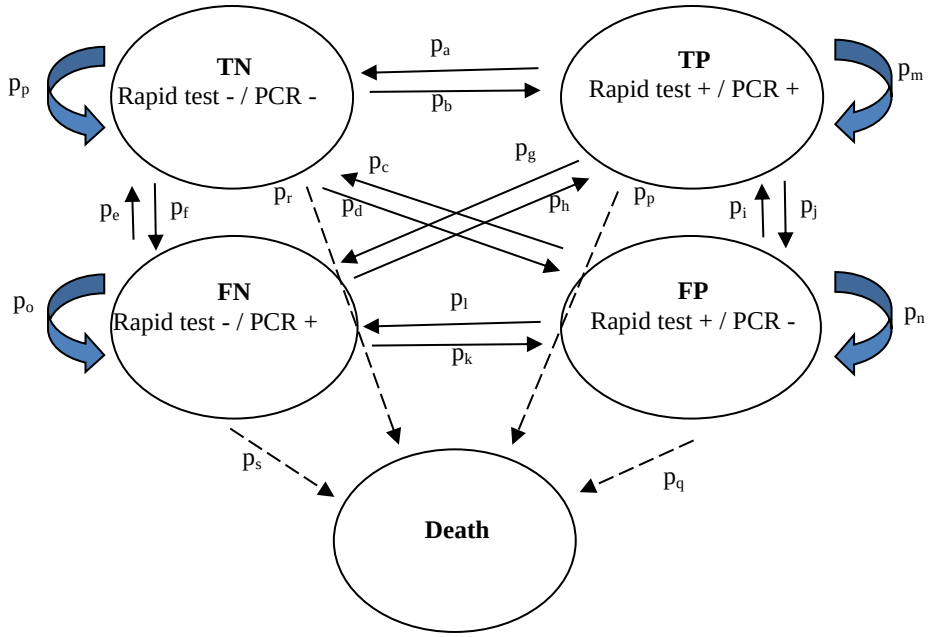
Two trials of antiretroviral prophylaxis in the first two years of life among infant in Malawi, a meta-analysis among HIV-exposed infants in multiple African countries, and our data found incident levels of 1.6% to 9.4% between birth and 3 months and levels of 2.8% and 4.9% between 6 and 12 months of age[133,134]. If the goal of any early infant diagnosis program is to timely identify new cases and promptly initiate treatment, testing timepoints corresponding to the highest levels of incidence are ideal. However, rapid testing at 3 months will result in most infants testing false positive since the majority of maternally-acquired antibodies have not waned by this time. Thus, we chose the next testing timepoint of 6 months

as the first timepoint. We chose 12 months, or 6 weeks after breastfeeding cessation, as the second timepoint as this corresponds to another time of highest risk of HIV acquisition in the postpartum period.

We also evaluated a testing strategy with timepoints 6 months and 15 months. Women are encouraged to exclusively breastfeed for 6 months and continue through 12 months. The 15 month timepoint would then allow women 6 weeks of breastfeeding cessation, the recommended time between cessation of breastfeeding and infant HIV testing.

4.3.3 Model structure

Using Markov modeling we simulated the economic and clinical outcomes of a hypothetical cohort of 10,000 HIV-exposed, uninfected infants from 6 weeks postpartum through the first 18 months of life as well as over a lifetime horizon. The 6 week time point was chosen as the starting point since HIV-exposed infants are to be tested with a PCR test at 4 to 6 weeks of age according to WHO EID testing guidelines[10]. We defined the follow-up period in 3 month cycles, beginning at 3 months of age and extending through 18 months. The model structure over a lifetime horizon was stratified as 0 to 2 years, 2 to 5 years, 5 to 10 years, 10 to 20 years, and 20 to 60 years. Figure 5 shows the health states a child could occupy during each three month cycle and the transition probabilities of moving to another health state or remaining in the same health state.



*FN-false negative; FP-false positive; TN-true negative; TP-true positive

Health states were mutually exclusive and defined as the POC test result at each time point, including false negative, false positive, true negative, or true positive results. A false negative result was defined as testing negative on the POC test but testing positive on HIV DNA PCR test. A false positive result was defined as testing positive on a POC test but testing negative on HIV DNA PCR test. A true negative result was defined at testing negative on a POC test and a HIV DNA PCR test. A true positive result was defined as testing positive on a POC test and a HIV DNA PCR test. Age-specific risk of death was modeled at every time period and was considered an absorbing health state. We defined the transition probabilities of moving from one health state to the other according to input parameters at each time point.

Figure 5. Model structure

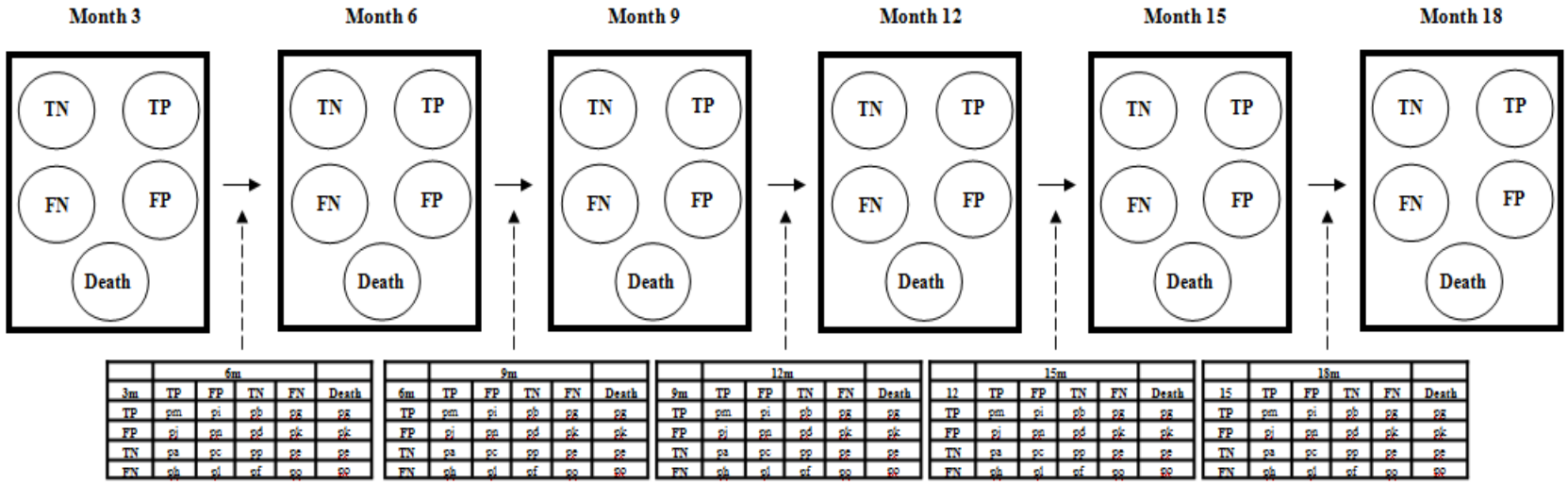
Health states were mutually exclusive and defined as the POC test result at each time point (false negative, false positive, true negative, or true positive results), using the result of the HIV DNA PCR test as the reference standard. The transition probabilities of going from one state to another were allowed to vary over the breastfeeding period, according to data from 3-month time cycles, which required repeated Markov structures specific to each 3-month cycle. Age-specific risk of death was modeled at every time period and was considered an absorbing health state. The transition matrix consisted of 20 probabilities, per time cycle, and is summarized in Table 10.

Table 10. Markov model transition matrix

	State (Time 2)				
State (Time 1)	**State A: TP**	**State B: FP**	**State C: TN**	**State D: FN**	**Death**
State A: TP	p_m	p_i	p_b	p_g	p_t
State B: FP	p_j	p_n	p_d	p_k	p_u
State C: TN	p_a	p_c	p_p	p_e	p_v
State D: FN	p_h	p_l	p_f	p_o	p_w

FP: false positive; FN: false negative; TP: true positive; TN: true negative

As a central assumption of Markov models, transition probabilities are assumed to be constant over time. However, in our analyses we did not expect the transition probabilities between states to remain constant over the breastfeeding period. Seroreversion occurs more rapidly in the first few months after birth, affecting both the sensitivity and specificity of each rapid test. Thus, the transition probabilities of going from one state to another state were assumed to not be the same over the breastfeeding period. Thus, Markov models and corresponding transition matrices for each 3 month time cycle were built (Figure 6).



*FN-false negative; FP-false negative; TN-true negative; TP-true positive

Figure 6. Markov model and transition matrices for each 3 month time cycle

4.3.4 Model input parameters for the base case model: Clinical input parameters (Table 11)

Overview

Where possible and relevant, we based clinical input parameters on data from a cohort of HIV-exposed infants who were HIV PCR negative (version 1.5 of the Amplicor HIV-1 DNA test kit, Roche, Basel, Switzerland) at age 6 weeks (median age 6.4 weeks, range 10.6 weeks), and in whom we measured the performance of HIV serological rapid tests. The study took place between 2008 and 2012 at two healthcare centers in the Blantyre region of Malawi. During the study period, PMTCT programs provided HIV counseling and testing to all pregnant women and offered single dose nevirapine or zidovudine for PMTCT. We prospectively collected data on 121 HIV-exposed infants, of which half (51%) were female and 80% received nevirapine at birth, which is similar to the current PMTCT uptake in Malawi seen in other studies, even in the Option B+ era[149,150]. Exclusive breastfeeding declined throughout the postpartum period, from 54% at month 3, to 18% at month 6, 1% at months 9 and 12, and 0% at months 15 and 18. Any breastfeeding declined from 87% of mothers at month 3, to 69% at month 6, 45% at month 9, 39% at month 12, 26% at month 15, and 17% at month 18. Maternal antiretroviral treatment (ART) use prior to birth was low (29%) and postpartum ART use and adherence was not recorded. We therefore based maternal ART use and adherence on published literature from sub-Saharan Africa and assumed that 88% of breastfeeding women will use ART at 6 weeks postpartum[151], 80% at 3 months postpartum[152] and 56% of these women will be adherent to ART at 12 months postpartum[147,152,153].

Our model structure was stratified according to age (0 to 2 years, 2 to 5 years, 5 to 10 years, 10 to 20 years, and 20 to 60 years). Mortality estimates, life expectancies, disability weights, and duration of HIV infection were defined for each time period and POC test result (i.e., true positive, true negative, false positive, or false negative). The observed mortality estimates in the first 24 months of life among HIV positive and HIV negative infants in our cohort correspond to what is found in the existing literature[9,25,153,154]. Thus, mortality estimates by age and by rapid test result were obtained from our data and the existing literature. For each infant, the most recent rapid test result prior to death was used to calculate the transition probability from the rapid test result at that time point to death in the following

time cycle. Mortality estimates for HIV-exposed, uninfected infants after 24 months were based on 2013 WHO Global Health Observatory Data Repository estimates for Malawi and a pooled analysis of several studies in Africa that included HIV-exposed, uninfected infants[131,155]. The average age of death for HIV infected infants age 24 months and older were based on a pooled analysis of 12 studies examining age-specific survival[154]. The average age of death was assumed to be the midpoint of the time period. Life expectancies for HIV infected and HIV-exposed, uninfected infants were based on a study in South Africa that had 56% PMTCT uptake, 87% linkage to postnatal care, Option B+ provision, and 36% pediatric ART adherence, which mimics the current reality in Malawi[156]. Disability weights associated with HIV/AIDS were estimated from the Global Burden of Diseases, Injuries, and Risk Factors Study 2010 (GBD)[157-159], which assigned a disability weight of 0.12 to diagnosed HIV. A disability weight of 0.50 was assigned to undiagnosed HIV, assuming these infants did not receive treatment and rapidly progressed to AIDS. A disability weight of 0.01 was assigned to persons uninfected with HIV. The duration of disease a person contributed within each time period was the number of years within that time period, assuming they lived to the end of the period. The duration of disease among persons who died was half of the time of that interval, assuming death occurred at the midpoint of the respective period.

Details of clinical input parameters

1. Mortality estimates, by age and rapid test result

Birth to 2 years

Background and input parameters

HIV positive: true positive on rapid test: Among infants infected with HIV during the postpartum period, no study has reported mortality risks based on whether the infant tested positive or negative according to a rapid test. Therefore, we based the mortality estimates within the first two years for HIV infected infants who tested positive from a rapid test on our data. In our data, 20% of infants who tested positive, thereby defining a true positive rapid test result, died by year 2. However, this estimate is likely overestimated given these infants used single dose nevirapine and did not have access to PI-based ART, a

combination that can lead to poor outcomes due to resistance. Therefore, we lowered the mortality estimate to 15%.

HIV positive: false negative on rapid test or missed on rapid test: A Zimbabwean study found 16% of infants infected postpartum died before 12 months and an additional 16% between 12 and 24 months[160]. Additionally, a pooled analysis of 12 studies with known timing of pediatric HIV infection found mortality among infants with postnatal HIV infections was 38% at year 2[154]. We can assume most of the infants did not receive any type of treatment in the 12 studies as most of the studies analyzed were conducted prior to widespread distribution of ART. In our data, 35% of infants who tested false negative died during-follow-up.

HIV-exposed, uninfected: Among infants not infected with HIV, mortality rates were based on for a pooled analysis of several studies in Africa that included HIV-exposed, uninfected infants[131].

Input parameters

Based on our data, we assumed mortality risks of 20% among HIV-exposed, infected infants who tested positive on a rapid test and 38% among HIV-exposed, infected infants who tested negative on a rapid test or were a missed infection. We assumed mortality of 8% among infants who were exposed, but uninfected.

Sensitivity parameters

HIV positive: true positive on rapid test: Sensitivity analyses were conducted around the mortality parameter for 0-2 year old HIV positive infants who tested positive (15%). The lower bound was calculated by assuming all infants received ART directly after diagnosis, which can reduce mortality by 75% if initiated early. The upper bound was based on a recent meta-analysis of 12 studies with known timing of pediatric HIV infection. Mortality among infants with postnatal HIV infections was 38% at year 2[154]. We can assume most of the infants did not receive any type of treatment in the 12 studies as most of the studies analyzed were conducted prior to widespread distribution of ART. The mortality proportion of 38% is similar to mortality of infants in our study who were a false negative by a rapid test, thus

hindering any type of treatment. Thus, the upper bound was set at 40% and assumed a majority of infants did not receive treatment.

HIV positive: false negative on rapid test, missed infection or delayed diagnosis: Sensitivity parameters were conducted around the mortality parameters for infants that tested false negative and infants that had missed infections. We did not expect the lower bound to be reduced dramatically for these infants as disease progression in infants occurs very rapidly[25,26]. However, in the Option B+ era, women taking HAART will deliver in better health (i.e., increased CD4 counts), which is associated with improved survival of their infants[131]. Thus, the lower bound was set at 15% to reflect improved health of mothers on HAART. The upper bound was calculated based on previous research showing that up to 60% of infants infected with HIV die by the age of 2 if untreated[25,26]. However, this estimate was lowered to 45% since the previous literature included both perinatal and postnatal infections and mortality among perinatally infected infants is higher than postnatally infected infants[25,43,154].

2 to 5 years

Background

HIV positive, true positive on rapid test: Mortality estimates attributable to HIV are limited and are usually aggregated for ages 0 to 14 years. In a pooled analysis examining age-specific survival in 12 studies that collected pediatric HIV mortality data, Marston and colleagues estimated a cumulative mortality among postnatally-infected infants by year 5 at 42%[154]. Thus, the proportion of infants dying with the first 2 years of life was estimated at 38% with the additional 4% dying between years 2 and 5. If we assume the cumulative mortality by year 5 is similar in our study, where 20% died in the first two years of life, an additional 22% would die between years 2 and 5. The cumulative mortality in the Marston study, however, was among studies without widespread provision of ART. Thus, we assumed the cumulative mortality in our study was lower due to the provision of ART.

HIV positive, false positive on rapid test: We did not expect false positive results from rapid test since the majority of placentally-acquired antibodies will have waned by 2 years.

HIV positive, missed infection or delayed diagnosis: Among children who but had a delayed or missed diagnosis, we assumed mortality to be lower after 2 years of age since disease progression slows after infancy[25,26].

HIV-exposed, uninfected: Among infants not infected with HIV, mortality rates were based on 2013 WHO Global Health Observatory Data Repository estimates for Malawi and a pooled analysis of several studies in Africa that included HIV-exposed, uninfected infants[131,155].

Input parameters

We assumed 10% of HIV positive infants who tested rapid test positive, 30% of HIV positive with missed or delayed infections, and 1% of HIV negative infants died between years 2 and 5 of age.

Sensitivity

Since data is limited, sensitivity parameters were assumed. The lower mortality bound was estimated assuming treatment was promptly initiated among HIV positive infants who tested rapid test positive. The upper bound was estimated assuming a minimal percentage of HIV positive infants initiated treatment.

5 to 10 years

Background

HIV positive, true positive on rapid test: In a meta-analysis examining age-specific survival in 12 studies that collected pediatric HIV mortality data, Marston and colleagues estimated mortality by year 10 at 53%[154]. The proportion of infants dying with the first 5 years of life was estimated at 42% with the additional 11% dying between years 5 and 10. Since most of the studies in the pooled analysis did not provide child PMTCT, we assumed morality among ages 5 to 10 years was lower than 11%.

HIV positive, missed infection or delayed diagnosis: Among children who a delayed or missed diagnosis, we assumed mortality to be lower after 2 years of age since disease progression slows after infancy, but similar to mortality during younger ages of 2 to 5 years[25,26].

HIV-exposed, uninfected: Among infants not infected with HIV, mortality rates were based on 2013 WHO Global Health Observatory Data Repository estimates for Malawi and a pooled analysis of several studies in Africa that included HIV-exposed, uninfected infants[131,155].

Input parameters

We assumed 8% of HIV positive infants who tested rapid test positive, 30% of HIV positive who with delayed or missed infections, and 0.8% of HIV negative infants died between ages 5 and 10.

Sensitivity

Since data is limited, sensitivity parameters were assumed. The lower mortality bound was estimated assuming treatment was promptly initiated among HIV positive infants who tested rapid test positive. The upper bound was estimated assuming a minimal amount initiated treatment and was similar to the estimate in the Marston pooled analysis.

10 to 20 years

Background

HIV positive, true positive: Marston and colleagues estimated mortality by year 20 at 84%[154]. The proportion of infants dying with the first 10 years of life was estimated at 53% with the additional 31% dying between years 10 and 20. Since most of the studies in the pooled analysis was prior to the implementation of Option B+ and prior to the availability of resistance testing and second and third line treatment regimens, we assumed morality among ages 10 to 20 years was lower than 31% since a higher majority of HIV positive persons initiate and adhere to treatment due to Option B and Option B+.

HIV positive, missed infection or delayed diagnosis: Among children acquiring HIV infections between ages 10 to 20 years, we assumed mortality to be lower after 2 years of age since disease progression slows after infancy, but slightly higher to mortality during younger ages of 2 to 10 years[25,26]. We increased mortality during this time period among HIV positive persons who tested rapid test negative or were a missed infection to correspond with the increase seen among HIV positive persons who tested rapid test positive.

HIV-exposed, uninfected: Among infants not infected with HIV, mortality rates were based on 2013 WHO Global Health Observatory Data Repository estimates for Malawi and a pooled analysis of several studies in Africa that included HIV-exposed, uninfected infants[131,155].

Input parameters

We assumed 20% of HIV positive infants who tested rapid test positive, 35% of HIV positive with missed infections or delayed diagnoses, and 0.8% of HIV negative children died between ages 5 and 10.

Sensitivity

Since data is limited, sensitivity parameters were assumed. The lower mortality bound was estimated assuming treatment was promptly initiated among HIV positive infants who tested rapid test positive. The upper bound was estimated assuming a minimal amount initiated treatment.

20 to 60 years

Background

HIV positive, true positive: Marston and colleagues estimated mortality by year 20 at 84%[154]. The proportion of infants dying with the first 20 years of life was estimated at 84% with the additional 16% dying between years 20 and 60. However, we assumed the cumulative mortality to be lower since the studies in the pooled analysis were prior to widespread distribution of treatment. The assumed cumulative mortality from birth to 20 years for our analysis was 64% with the additional 36% of persons dying by age 60.

HIV positive, missed infection: We assumed mortality to be lower after 2 years of age since disease progression slows after infancy, but slightly higher than mortality during younger ages of 2 to 20 years[25,26].

HIV-exposed, uninfected: Among infants not infected with HIV, mortality rates were based on 2013 WHO Global Health Observatory Data Repository estimates for Malawi and a pooled analysis of several studies in Africa that included HIV-exposed, uninfected infants[131,155].

Input parameters

We assumed 36% of HIV positive infants who tested rapid test positive, 35% of HIV positive persons were missed infections or delayed diagnoses, and 15% of HIV negative persons died between ages 20 and 60.

Sensitivity

Since data is limited, sensitivity parameters were assumed. The lower mortality bound was estimated assuming treatment was promptly initiated among HIV positive infants who tested rapid test positive. The upper bound was estimated assuming a minimal amount initiated treatment.

2. Life expectancy, average age of death, and number of years lost by age and rapid test result

Life expectancy

Background and input parameters

Life expectancy for all infants, regardless of HIV infection, in Malawi was based on the 2013 WHO Global Health Observatory Data Repository estimates for Malawi[155]. Life expectancy, in years, was stratified by ages of less than 2 years, 2 to 5 years, 5 to 10 years, 10 to 20 years, and 20 to 60 years.

Average age of death

0 to 2 years

Background and input parameters

The average age of death for HIV positive infants less than 2 years of age was based on our data and the average age of death for HIV negative infants less than 2 years of age was assumed to be 1 year, based on the midpoint of the time period. The average age of death for HIV positive infants was based on our data and previous research evaluating cost-effectiveness and clinical outcomes of several WHO recommended PMTCT strategies in Zimbabwe[161]. The cohort of pregnant, HIV infected women resembled our cohort of women with an average age of 24 years and average breastfeeding duration of 15 months. The Zimbabwe study examined several PMTCT strategies, included Option B, Option B+, ART provision and adherence to the infant after pediatric infection, and linkage to care during the postpartum

period. The average age of death was assumed to be 0.9 months for HIV positive infants and 12 months for HIV negative infants.

2 to 5 years and 5 to 10 years

The average age of death for children older than 2 but less than 10 was based on a previous study in South Africa[156]. The average age of death of 21.41 years was based on an assumption of 56% PMTCT uptake, 87% linkage to postnatal care, Option B+ provision, and 36% pediatric ART adherence, which mimics the current reality in Malawi.

10 to 20 years and 20 to 60 years

It is of no surprise that ART can dramatically affect life expectancy in HIV positive persons. A Ugandan study among 22,315 patients receiving combination ART found life expectancy increased by 26.7 (25.0, 28.4) years if ART was initiated at age 20 and by 27.9 (26.7, 29.1) years if ART was initiated at age 35[162]. Although, the study did not provide estimates of ART adherence during the follow-up period, 59% of persons initiated ART at WHO stage 2 and 30% initiated ART at WHO stage 3. Thus, we assume treatment was not initiated directly after infection but rather after the acute infection period was over in the majority of patients. A more recent modeling study was conducted among HIV infected patients who were older than 15 years and treatment naïve in South Africa[163]. The average life expectancy among patients who initiated ART at age 25 years was increased 31.4 years (26.7, 36.2) and was 25.9 (22.2, 30.0) if ART was initiated at age 35 years. Similar to the Ugandan study, ART adherence was not provided in the South African study. The Malawi HIV epidemic resembles the Ugandan epidemic more closely than the South African epidemic, where provision of treatment is more widespread. Assuming ART adherence will likely continue to increase in low income countries, due to widespread dissemination and provision of treatment, we assumed an average duration of 27 years for adults ages 10 to 20 years and 24 years for adults older than 20 years, similar to the Ugandan study.

3. Disability weights

Background

Disability weights associated with HIV/AIDS were estimated from the Global Burden of Diseases, Injuries, and Risk Factors Study 2010 (GBD)[157-159]. In the 2010 GBD study, a disability weight of 0.12 was assigned to undiagnosed HIV without symptoms. A disability weight of 0.50 was assigned to undiagnosed HIV, assuming these infants did not receive treatment and rapidly progressed to AIDS.

Input parameters

Assuming most infants will be diagnosed without symptoms, otherwise the infants would have been brought in for early testing, a disability weight of 0.12 was assigned to infants who tested true positive. A disability weight of 0.50 was assigned to infants who tested false negative or were missed infections, on the assumption they would be untreated. A disability weight of 0.01 was assigned to HIV-exposed, uninfected infants (i.e., infants who tested false positive and true negative on a rapid test).

4. Number of incident cases

Background and input parameters

The incident number of cases by rapid test result type was calculated according to our data from Malawi. The transition probabilities for each 3 month cycle was used to estimate the number of cases if an infant tested true positive or false negative according to a rapid test in the cohort of 10,000 infants.

The incident number of cases for HIV-exposed, negative infants (i.e., those that tested false positive and true negative on a rapid test) between ages 0 to 2 years was calculated based on our data, with 17% of infants becoming infected during that time period. The incident number of cases for all other age ranges was based on previous pooled data from sub-Saharan Africa[161,164].

Sensitivity parameters

Among HIV-exposed, uninfected infants, we explored the lifetime incidence rate assumption through sensitivity analyses with a range of 2% to 50% to account for dissemination of pre-exposure prophylaxis, contributing to a lower lifetime risk, and high-risk behaviors, contributing to a higher lifetime risk of HIV.

5. Duration of disease (years), by age

Our model structure was stratified by time periods throughout a person's life (i.e., 0 to 2 years, 2 to 5 years, 5 to 10 years, 10 to 20 years, and 20 to 60 years. Thus, the duration of disease a person contributed within each time period was the number of years within that time period, assuming they lived to the end of the period. If a person died within that interval, we assumed the death occurred in the middle of the period. Thus, the duration of disease among persons who died was half of the time for that interval.

4.3.5 Model input parameters for the base case model: Economic input parameters (Table 11)

Overview

The cost of a Determine and Unigold test was set at $1.50 and $5.00, respectively. An additional $1.60 was added to each serological rapid test for sample preparation costs and personnel. The cost of the Alere Q HIV test was set at $25.00 per test and included equipment costs, sample preparation and personnel[165]. The costs of a PCR was set at $35.00 and included costs of the tests, sample preparation, and personnel[166]. HIV pre- and post-test counseling costs were determined based on research conducted in Uganda[75]. The costs of providing ART to HIV infected children were based on cost-effectiveness research conducted in Rwanda[167].

The costs of a false positive POC test included the cost of the POC test, costs of pre- and post-test HIV counseling, cost of a follow-up PCR, and costs of counseling after a negative PCR test. The costs of a true positive POC test included the cost of the POC test, costs of pre- and post-test HIV counseling, cost of a follow-up PCR, costs of counseling after a positive PCR test, and treatment costs. The costs of a false negative and true negative POC test were the same and included the cost of the POC test and costs of pre- and post-test HIV counseling.

Details of economic input parameters

Testing costs

The costs of each rapid test and the costs associated with PCR were estimated based on our data sources in Malawi and previous research conducted in Uganda[75]. The costs of a Determine test was $1.50 and the costs of a Unigold test was $5.00. An additional $1.60 was added to each rapid test for sample

preparation costs and personnel. The costs of the Alere Q HIV test was estimated to be $25.00 per test and included equipment costs, sample preparation and personnel[165]. Each Alere Q HIV machine costs $10,000 and can perform 7,000 tests per year. The costs of a PCR was estimated at $35.00 and includes costs of the tests, sample preparation, and personnel[166].

Counseling costs

HIV pre and post test counseling costs were determined based on previous research conducted in Uganda[75]. Costs associated with a negative rapid test or a negative PCR test was $1.40 per test and costs associated with a positive rapid test or a positive PCR test was $1.50 per test.

Infant HIV infection costs

The costs of providing ART to HIV infected children were based on previous cost-effectiveness research conducted in Rwanda[167]. For children less than 12 months of age, the cost of treatment for each HIV infected infant was $558.32. For children older than 12 months of age, the cost of treatment for each HIV infected child was $527.52. For simplicity, the costs were combined for the follow-up period resulting in $542.92 per year for each infected children from 3 months to 18 months of age.

Test result costs

The costs of a false positive rapid test or false positive Alere PCR test included the cost of the point-of-care test, costs of pre and post test HIV counseling after a positive point-of-care test, cost of a follow-up PCR, and costs of pre and post test HIV counseling after a negative PCR test. The costs of a true positive rapid test or true positive Alere PCR test included the cost of the point-of-care test, costs of pre and post test HIV counseling after a positive point-of-care test, cost of a follow-up PCR, costs of pre and post test HIV counseling after a positive PCR test, and treatment costs for the infected infant. The costs of a false negative rapid test or false negative Alere PCR test included the cost of the point-of-care test and costs of pre and post test HIV counseling after a negative point-of-care test. The costs of a true negative rapid test or true negative Alere PCR test included the cost of the point-of-care test and costs of pre and post test HIV counseling after a negative point-of-care test.

Sensitivity parameters

The cost of providing ART varies from country to country. A recent cost-effectiveness analysis conducted among four countries found discounted costs for infant ART ranging from $801 in Zambia, $759 in Kenya, $404 in Vietnam, and $279 in South Africa[168]. Based on these estimates, we varied our costs of infant ART from $300 to $800. These ranges were incorporated in the total costs if the test result was a true positive.

4.3.6 Model input parameters for the base case model: Test performance parameters (Table 11)

In our cohort study, serological rapid HIV tests were performed at POC in Malawian HIV-exposed uninfected infants at ages 3, 6, 9, 12, 15, and 18 months postpartum at 6 weeks. At missing data points (approximately 30%), rapid tests were conducted on stored blood at a University of North Carolina research laboratory. To account for missing data due to unavailable stored blood and not collected in Malawi, we used multiple imputation methods using Markov chain Monte Carlo methods[169] and Proc MI in SAS to impute the missing values for five datasets[101,102]. HIV DNA PCR was considered the gold standard and performed at each time a rapid test was done. Confirmation of infant HIV infection detected by a positive HIV DNA PCR result was done through a second HIV DNA PCR assay or a HIV RNA viral load assay. Using HIV DNA PCR as the reference standard, we calculated the sensitivity and specificity of Determine and Unigold in 3 month intervals, from month 3 to month 18 postpartum (Table 2). The corresponding transition probabilities were averaged over the five datasets to create the final transition probabilities for each rapid test at each time cycle.

Transition probabilities between health states for the Alere q HIV1/2 test for each 3 month cycle were calculated based on test results from two African studies[170,171]. The sensitivity of the Alere test in a South African study of 1,098 infants with a median age of 47 days (IQR: 42-177 days) was 95.5% (95% CI: 91.7 to 97.9) and specificity was 99.8% (95% CI: 99.1 to 100.0) among all infants. Age-specific estimates were not provided[45]. The sensitivity and specificity of the Alere test in a Mozambique study of 827 infants with a median age of 42 days (IQR: 30-90 days), were 97.3% (95% CI: 85.8 to 99.9) and 99.9% (95% CI: 99.2 to 100.0), respectively, among infants younger than 6 months and was 99.9% (95%

CI: 76.8 to 100.0) and 100.0% (95% CI: 92.1 to 100.0) among infants age 6 to 18 month[46]. Based on these two studies, we used values for sensitivity and specificity of 97.0% and 99.8%, respectively, between months 3 and 9, and sensitivity and specificity values of 99.0% and 100.0%, respectively, between months 9 and 18 to calculate the transition probabilities.

Table 11. Model input and sensitivity parameters

Parameters	Base-case value	Sensitivity parameters	Data source
Clinical model assumptions and input parameters			
Test performance			
Sensitivity			
Determine			
3 month	100.0	47.8, 100.0	
6 month	100.0	39.8, 100.0	
9 month	66.7	22.3, 95.7	
12 month	100.0	69.2, 100.0	
15 month	87.5	47.4, 99.7	
18 month	100.0	63.1, 100.0	Our data
Unigold			
3 month	100.0	47.8, 100.0	
6 month	100.0	39.8, 100.0	
9 month	75.0	19.4, 99.4	
12 month	81.8	48.2, 97.7	
15 month	87.5	47.4, 99.7	
18 month	100.0	63.1, 100.0	
Alere			
3 month-9 month	97.0	91.7, 100.0	[170], [171]
12 month-18 month	99.0	91.7, 100.0	
Specificity			
Determine			
3 month	7.0	2.3, 15.7	
6 month	34.4	24.8, 44.9	
9 month	46.5	35.9, 57.1	
12 month	88.3	81.1, 95.5	
15 month	95.6	90.7, 100.0	
18 month	94.6	88.8, 100.0	
Unigold			Our data
3 month	19.4	11.1, 30.5	
6 month	83.7	74.4, 89.9	
9 month	97.7	94.6, 99.7	
12 month	100.0	95.4, 100.0	
15 month	100.0	94.5, 100.0	
18 month	100.0	94.0, 100.0	
Alere			
3 month - 9 month	99.8	99.1, 100.0	[170], [171]
12 month - 18 month	99.9	99.1, 100.0	

Parameters needed to calculate DALYs											
	0-2	**2-5**	**5-10**	**10-20**	**20-60**	**0-2**	**2-5**	**5-10**	**10-20**	**20-60**	
Mortality estimates (%)											
HIV positive, TP	15	10	8	20	36	4, 40	4, 16	5, 11	10, 31	31, 41	[154], our data
HIV positive, FN	38	-	-	-	-	15, 45	-	-	-	-	[154], our data

HIV positive, missed	38	30	30	35	35	20, 40	20, 40	15, 35	25, 45	25, 45	[154], our data
HIV negative, FP or TN	8	1	0.8	0.8	15	-	-	-	-	-	[172]
Life expectancy											
HIV positive, TP	20.51	18.91	13.91	27.0	27.0	-	-	-	-	-	[155,161-163]
HIV positive FN or missed	20.51	32.50	27.50	45.00	27.00	-	-	-	-	-	
Number of incident cases (%)	17	2	2	30	1	2, 25	1.8, 3.0	1.8, 3.0	10, 45	0.9, 1.5	[161,164]
Duration of HIV/AIDS (years)	2	3	5	10	40	-	-	-	-	-	
Economic model input parameters											
Testing costs (includes test and sample prep)											
Determine			$3.10			-			Our data, [75]		
Unigold			$6.60			-			Our data, [75]		
Alere PCR			$25.00			-			[165]		
PCR			$35.50			-			Our data, [166]		
Counseling costs											
Pre/post with negative result			$1.40			-			[75]		
Pre/post with positive result			$1.50			-			[75]		
Costs for HIV infected child											
ART per year			$542.92			$300-$800			[167]		
Costs associated with test results											
False positive Determine			$41.00			-			Our data, [75,166]		
False positive Unigold			$44.50			-			Our data, [75,166]		
False positive Alere			$72.90			-			[165,166]		
True positive Determine			$584.02			$341.10-$841.10			Our data, [75,166,167]		
True positive Unigold			$587.52			$344.60-$844.60			Our data, [75,166,167]		
True positive Alere			$615.92			$373.00-$873.00			[165-167]		
False negative Determine			$4.50			-			Our data, [75]		
False negative Unigold			$8.00			-			Our data, [75]		
False negative Alere			$36.40			-			[165]		
True negative Determine			$4.50			-			Our data, [75]		
True negative Unigold			$8.00			-			Our data, [75]		
True negative Alere			$36.40			-			[165]		

*ART-Antiretroviral treatement; EBF-Exclusive breastfeeding; MTCT-Mother-to-child transmission; NVP-Nevirapine; PMTCT-prevention of mother to-child transmission; PCR-polymerase chain reaction

Note: Costs assumed a triangular distribution and all other parameters assumed beta distributions.

4.3.7 Outcomes

We projected the clinical and economic outcomes of each strategy, and the incremental cost-effectiveness. Economic outcomes included testing costs, HIV pre- and post-test counseling costs, healthcare and treatment costs, and total costs.

Clinical outcomes included missed pediatric HIV infection, number of false negative, false positive, true negative, and true positive rapid test results, and Disability Adjusted Life Years (DALYs). A missed pediatric HIV infection was defined as any infection that occurred but was not detected by the HIV rapid test used as well as any infection occurring six months or more before the testing time point, as both negatively impacts the benefits of early treatment initiation. For example, missed infections for the EID strategy 7 (test once at 9 months with Determine) would include all infections after 9 months and any infections between age 6 weeks and 3 months but not identified by Determine at the 9 month testing time point. DALYs were expressed as the number of years lost due to early death, disability, or ill-health, thereby combining mortality and morbidity into a single metric[173,174]. Years of life lost (YLL) to an HIV infection was calculated from the number of deaths in the population, the standard life expectancy at age of death, and a discount rate[174].

$$YLL = \frac{N}{r}(1 - e^{-rL})$$

where:
N = number of deaths.
L = standard life expectancy at age of death (years).
r = discount rate (e.g. 3% corresponds to a discount rate of 0.03).

Years of life lost to disability (YLD) from HIV infection was calculated from the number of incident cases, disability weights, duration of disease, and a discount rate[174].

$$YLD = \frac{I \times DW \times L\,(1-e^{-rL})}{r}$$

where:
I = number of incident cases (-).
DW = disability weight (-).
L = duration of disability (years).
r = discount rate.

YLL and YLD were calculated for each age group and for each health state. Health benefits were discounted at 3% annually throughout the lifetime horizon[138].

We assessed the relative value of each EID strategy to the next most expensive strategy using the incremental cost-effectiveness ratio (ICER) by ranking all EID strategies in sequential order by total costs[175,176]. The incremental effectiveness of each EID strategy was evaluated as DALYs averted, compared with the next most expensive strategy. Strategies were considered dominated (i.e., cost-effective) if DALYs were not averted in the next most expensive strategy. After the initial comparisons of all strategies in sequential order, dominated strategies were eliminated from the ranking, and the incremental effectiveness of the remaining strategies were evaluated again as DALYs averted, compared with the next most expensive strategy. This iterative process continued until all remaining strategies were undominated by the next most expensive strategy, resulting in the final ICER calculations. ICER results from the base case were compared with a willingness-to-pay threshold of 1 or 3 times the per capita Malawi gross domestic product (GDP) per DALY averted. The WHO considers interventions that cost 1 to 3 times the per capita GDP per DALY averted as cost-effective, with interventions that costs less than 1 times the per capita GDP considered very cost-effective[177,178].

CHAPTER 5: RESULTS. PERFORMANCE OF HIV RAPID TESTS AMONG BREASTFEEDING, MALAWIAN INFANTS

In the past decades, large gains have been made in reducing the burden of infant HIV infection through implementation of effective prevention of mother-to-child transmission (PMTCT) programs, primarily in the prenatal and delivery period[19,221]. However, implementation of the 2010 World Health Organization (WHO) breastfeeding guidelines may shift the timing of infant HIV infection to the postnatal period as HIV positive mothers are now encouraged to exclusively breastfeed for the first 6 months of life and continue breastfeeding for at least the first 12 months of life, increasing the duration of the infant's recurrent exposure to HIV compared to the previous guidelines[8]. In areas with a tradition of prolonged breastfeeding, it is estimated that as many as 8 out of 10 pediatric HIV infections may occur during the breastfeeding period[1].

The WHO recommends HIV-DNA/RNA polymerase chain reaction (PCR) and ultra-sensitive p24 antigen assays for diagnosis of infant HIV infections[10]. These tests, however, are limited in resource poor settings by logistical and financial constraints and high loss to follow-up during the breastfeeding period since mothers are required to return for test results[11,12]. Rapid HIV tests are cheaper and allow for quick results (i.e., 20 minutes) but these tests detect HIV antibodies and the presence of circulating placentally-transferred maternal HIV antibodies, complicating their interpretation in young infants[13,14]. The time of seroreversion, or decay of maternal antibodies, ranges between 9 to 18 months, with the most significant decay occurring by 12 months of age[17,73,107-110]. The WHO therefore recommends rapid tests as a screening assay to determine HIV exposure in children less than 18 months of age or as a diagnostic assay in children older than 18 months of age[27].

In previous studies of the persistence of maternal HIV antibodies among infants younger than 18 months of age, children were aggregated regardless of age and were mainly comprised only infants older

than one year[13,15-17]. Thus, the utility of rapid tests in infants in high HIV burden settings during early infancy and throughout the postpartum period, particularly in relation to the decay of placentally-acquired antibodies, is not yet well understood. We aimed to evaluate the performance of two commonly used rapid tests in the public sector at 6 timepoints during the first 18 months of life in Malawian, breastfed HIV-exposed children. A detailed description of the methods is available in section 4.1 of this document.

5.1 Results

Overall, 121 HIV-exposed infants contributed 996 HIV rapid test results between 3 months and 18 months of age. Of the 996 rapid tests, 295 Determine and 294 Unigold were conducted in real-time in Malawi; 197 Determine and 210 Unigold were conducted at UNC. The 3 month tests were performed almost exclusively (99%) at UNC on stored blood. Approximately two-thirds of tests at the 6, 9, and 12 month timepoints were performed in real-time in Malawi. After 12 months, over 75% of all tests were performed in real-time in Malawi.

Of the 121 mother-infant pairs, half of the infants were female, 98 (81%) of the infants received single dose nevirapine at birth, and 24 (20%) of the mothers reported taking daily antiretrovirals prior to birth (Table 12). Most (69%, 84/121) infants breastfed up to 6 months, but only 22 (18%) infants were exclusively breastfed through month 6. Of the 21 infants infected during follow-up, 9 (43%) were still breastfeeding at 18 months; only 17 of the 100 (17%) non-infected infants were still breastfeeding at 18 months.

Among the cohort of 121 HIV-exposed infants who were HIV negative at age 6 weeks, 21 became infected, corresponding to a cumulative incidence of HIV infection of 17.2% (95% CI: 11.1, 24.5) over 18 months. Of the 21 infants who became infected with HIV between 6 weeks and 3 months, 1.6% of infants became infected between birth and 3 months, 4.1% between 3 and 6 months, 0.8% between 6 and 9 months, 5.0% between 9 and 12 months, 4.9% between 12 and 15 months, and 0.8% between 15 to 18 months.

Excluding HIV infection

The specificity estimates of both tests increased with infant age, but the estimated specificity of the Unigold assay was higher than that of the Determine assay at all ages (Figure 7, Table 13). For Unigold, the estimated specificity increased from 19.4% (95% CI: 11.1, 30.5) at 3 months to 83.7% (74.4, 89.9) at 6 months, reaching 100 (95% CI: 95.4, 100.0) by age 12 months. The specificity estimates of the Determine test increased from 7.0% (2.3, 15.7) at 3 months to 34.4% (24.8, 44.9) at age 6 months, leveling off at 95.6% (90.7, 100.0) by 15 months of age.

Diagnosing incident HIV infection

The estimated sensitivity, defined as true incident HIV infection, of both tests displayed a U-shaped curve with 100% sensitivity before age 6 months, low sensitivity (around 70%) at age 9 months, after which sensitivity increased again, reaching 100% for both rapid tests at age 18 month. Due to the low event rates, sensitivity estimates were imprecise, with broad 95% confidence intervals (Table 13).

Among the 21 infants, we observed 4 different infection scenarios: (1) incident HIV infection prior to seroreversion, (2) seroreversion followed by detection of an incident infection by a rapid test, (3) incident HIV infection with false negative HIV rapid test due to the window period, and (4) all other scenarios with insufficient information necessary to characterize (Figure 8). The first scenario, incident infection prior to seroreversion, occurred in six children (A-F). The second scenario, seroreversion followed by detection of an incident infection by a rapid test, occurred in four children (F-I) for Determine and in three children for Unigold (G-I). In all four children, incident infection occurred between 9 and 12 months. For three children (G-I), we were able to observe seroreversion followed by identification of an incident infection at age 12 months with both rapid tests. In one child (H), we observed seroreversion prior to incident infection for both tests, but identification of an incident infection was only documented by Determine. The third scenario, incident infection with a false negative rapid test most likely representing the window period, occurred in four children (H, J, K, L). In two children (J, L), incident infection occurred between 6 and 9 months of age, but either Determine (L) or both rapid tests (J)

were false negative at age 9 months. In the remaining nine children (M-U), insufficient data was available to classify them.

Positive and negative predictive values of rapid test results

The estimated PPV was low at most ages for both tests and decreased with decreasing population incidence of HIV infection (Table 14). The estimated PPV of Unigold was substantially higher than that of Determine at all ages except for age 3 months, an age at which the PPV was extremely low (≤ 7%) for both rapid tests. The estimated PPV for Determine never reached more than 52%, independent of age or population infant HIV incidence. An estimated PPV greater than 80% was reached for Unigold at age 12 months or older in settings with HIV incidence ≥5%.

The estimated NPV was very high (≥99%) for both rapid tests at all ages regardless of the population infant HIV incidence, except for age 9 months in settings with ≥4% risk of HIV acquisition, where the estimated NPV value of Determine was slightly lower (96-97%).

5.2 Discussion

Even in the era of option B+, monitoring infant HIV infection status in the breastfeeding period is critical for timely identification of HIV infection, rapid initiation of antiretroviral treatment and accurately ruling out HIV infection. For all HIV-exposed infants with negative virological tests at age 4 to 6 weeks, the WHO currently recommends a rapid test at 9 months and, if negative, again at 18 months or 6 weeks after the cessation of breastfeeding without providing guidance regarding what type of rapid test to use. We show that performance of rapid tests in young infants depends on the infant's age and the type of rapid test. Thus, recommendations for rapid testing should incorporate age-specific guidelines and information on what type of test to best use during the postpartum period.

At 3 months of age, rapid HIV tests have minimal clinical value since recent infection cannot be distinguished from circulating maternal antibodies. Between 6 and 12 months of age, however, screening by rapid tests could play a role in the follow up of HIV-exposed infants, but the clinical utility depends on the type of rapid test used, with Unigold outperforming Determine at these timepoints. At age 12 months and beyond, the type of test is less important, as both tests identifed HIV-free children, although both

tests failed to detect some incident infections. Finally, as we observed no difference in test performance between ages 15 to 18 months, it may be possible to change the current recommendation to test HIV-exposed, uninfected infants at 18 months to the earlier timepoint of 15 months. From a mother's perspective, this would reduce the stressful waiting time regarding her child's HIV status.

The "worse" performance of the Determine rapid test is not surprising as Determine is a more sensitive test compared to the Unigold test in adult populations[179,180]. The Determine test has a lower threshold for HIV antibodies than Unigold. Consequently, maternal antibodies will continue to be detected longer with Determine than Unigold. Thus, the proportion of false positives will be higher for Determine, reducing the specificity of Determine in this context. Although test sensitivity may have been inflated due to testing on stored blood, [17,73,181], we do not expect this to impact the results substantially as 60% of all testing was conducted in Malawi in real-time.

Our study was the first to prospectively compare two rapid tests at multiple timepoints, including very young ages, in a cohort of predominantly breastfeeding infants in a resource limited setting. Our results are consistent with previous studies[22,23] in regard to test sensitivity but not specificity, though comparisons with other data are difficult given the scarcity of rapid test performance data among young infants[17,73]. In mainly formula-fed South African children, the estimated specificity for Determine was 82% at 6 to 9 months and 100% at 9 to 12 months[73], compared to 46.5% at 9 months and 88.3% at 12 months in our cohort of mainly breast-fed Malawian children. Other studies evaluating tests performance aggregated all infants less than 18 months of age, making comparisons difficult[17,74]. . While many more rapid tests are commercially available and used in resource limited settings, we only evaluated those two that are commonly used in the public sector in Malawi and did not include any fourth generation rapid tests. One small study showed that fourth generation tests did not perform better in infants compared to third generation tests[80].

Based on our findings, the use of Unigold, or another rapid test with similar performance characteristics, throughout the entire postpartum period could be advocated for, particularly given the outperformance of Unigold at all timepoints, including the younger ages where timely identification of

infection and prompt treatment initiation is important. Although Determine is cheaper than Unigold, the lower percentage of false positives with Unigold would result in fewer unnecessary follow-up PCR tests, thereby reducing overall costs in an early infant diagnosis program. Another option for testing during this time period are point-of-care virological assays, which distinguish between true infections and placentally-acquired HIV antibodies at young ages. While preliminary data from studies in early infant diagnosis programs are promising, [170,171] the cost of these novel point-of-care virological assays are expected to be nearly seven times the costs of a rapid test[165], likely too expensive for repeated use during the first 18 months of life in the 1.5 million HIV-exposed children born annually in high burden countries[3]. It is therefore likely that a potential role for rapid tests in the repeat screening of HIV-exposed infants remains.

In conclusion, timely, accurate, and affordable testing algorithms at point-of-care are critical for effective early infant diagnosis in the postpartum period. Our findings that the performance of rapid tests differed by age, test type, and for different population infant HIV incidence levels, warrants evaluation of the use of rapid tests in large cohorts of HIV-exposed children. Updated guidelines for use of rapid tests in HIV-exposed infants that explicitly take timing and test type into account are urgently needed to ensure optimal care for the 1.5 million HIV-exposed infants born annually.

Table 12. Characteristics of cohort of HIV-exposed infants with negative PCR test at 6 weeks in Malawi

	All (N=121)	HIV-exposed, uninfected (N=100)	HIV-exposed, infected* (N=21)	
Infant characteristics	**N (%) Median (IQR) Mean (SD)**	**N (%) Median (IQR) Mean (SD)**	**N (%) Median (IQR) Mean (SD)**	**p-value**
Gender				
Female	61 (51)	49 (50)	12 (57)	
Male	60 (49)	51 (50)	9 (43)	0.53
Exclusive Breastfeeding status (month)				
3	66 (54)	56 (56)	10 (48)	
6	22 (18)	18 (18)	4 (19)	
9	1 (1)	1 (1)	0 (0)	
12	1 (1)	1 (1)	0 (0)	
15	0 (0)	0 (0)	0 (0)	
18	0 (0)	0 (0)	0 (0)	0.62
Any Breastfeeding status (month)				
3	106 (87)	87 (87)	19 (90)	
6	84 (69)	70 (70)	14 (67)	
9	55 (45)	40 (40)	15 (71)	
12	47 (39)	35 (35)	12 (57)	
15	32 (26)	24 (24)	9 (43)	
18	21 (17)	12 (12)	9 (43)	0.16
NVP at birth				
Yes	97 (80)	80 (80)	17 (81)	
No	6 (5)	6 (6)	0 (0)	
Unknown	18 (15)	14 (14)	4 (19)	0.26
Weight-for-age z-score (mean)	-1.05 (1.25)	-1.84 (1.10)	-0.69 (0.87)	NA
Maternal characteristics				
Age (median)	27 (IQR: 18-40)	27 (IQR: 18-40)	29 (IQR: 20-39)	0.65
Maternal ARV use prior to birth	87 (72)			
No	24 (20)	69 (69)	17 (81)	
Yes	10 (8)	22 (22)	1 (5)	
Unknown		9 (9)	3 (14)	0.08
Marital status				
Single (divorced, widowed)				
Married (married, living with partner)	17 (14)	12 (12)	2 (10)	
Unknown	99 (82)	85 (85)	17 (80)	
Education level	5 (4)	3 (3)	2 (10)	0.82
Completed ≥ secondary level	62 (51)			
Completed < secondary level	54 (45)	76 (76)	14 (66)	
Unknown	5 (4)	21 (21)	5 (24)	
		3 (3)	2 (10)	0.66

*HIV infection occurred after 6 weeks

Table 13. Sensitivity and specificity of Determine and Unigold assays during the postpartum period

	Results						
	Total tested	**True positive** (PCR+, rapid test+)	**False Negative (**PCR+, rapid test-)	**False positive** (PCR-,rapid test+)	**True negative (**PCR-, rapid test-)	**Sensitivity (95% CI)**	**Specificity (95% CI)**
Infant age	**N**	**n (%)**	**n (%)**	**n (%)**	**n (%)**		
3 months							
Determine	76	5 (7)	0 (0)	66 (86)	5 (7)	100.0 (47.8, 100.0)	7.0 (2.3, 15.7)
Unigold	77	5 (7)	0 (0)	58 (75)	14 (18)	100.0 (47.8, 100.0)	19.4 (11.1, 30.5)
6 months							
Determine	97	4 (4)	0 (0)	61 (63)	32 (33)	100.0 (39.8, 100.0)	34.4 (24.8, 44.9)
Unigold	105	4 (4)	0 (0)	17 (16)	84 (80)	100.0 (39.8, 100.0)	83.7 (74.4, 89.9)
9 months*							
Determine	92	4 (4)	2 (2)	46 (50)	40 (43)	66.7 (22.3, 95.7)	46.5 (35.9, 57.1)
Unigold	91	3 (3)	1 (1)	2 (2)	85 (94)	75.0 (19.4, 99.4)	97.7 (94.6, 99.7)
12 months							
Determine	88	10 (11)	0 (0)	9 (11)	68 (77)	100.0 (69.2, 100.0)	88.3 (81.1, 95.5)
Unigold	91	9 (10)	2 (2)	0 (0)	79 (88)	81.8 (48.2, 97.7)	100.0 (95.4, 100.0)
15 months							
Determine	76	7 (9)	1 (1)	3 (4)	65 (86)	87.5 (47.4, 99.7)	95.6 (90.7, 100.0)
Unigold	73	7 (10)	1 (1)	0 (0)	65 (89)	87.5 (47.4, 99.7)	100.0 (94.5, 100.0)
18 months							
Determine	64	8 (13)	0 (0)	3 (4)	53 (83)	100.0 (63.1, 100.0)	94.6 (88.8, 100.0)
Unigold	68	8 (12)	0 (0)	0 (0)	60 (88)	100.0 (63.1, 100.0)	100.0 (94.0, 100.0)

Abbreviations: CI, confidence interval;

*Of the 6 HIV incident infections detected at 9 months, 4 were tested with Determine and Unigold and 2 were tested only with Determine due to Unigold stock-out at the testing site.

Table 14. Positive and negative predictive values of Determine and Unigold assays during the postpartum period with varying pediatric HIV incident infections

Positive predictive value												
Incidence*	**3m**	**95% CI**	**6m**	**95% CI**	**9m**	**95% CI**	**12m**	**95% CI**	**15m**	**95% CI**	**18m**	**95% CI**
5%												
Determine	5.3	(0.0, 10.7)	7.4	(1.1, 13.8)	6.2	(0.0, 12.9)	31.0	(8.4, 53.7)	51.1	(20.2, 82.1)	49.4	(19.8, 78.9)
Unigold	6.1	(0.0, 12.1)	24.4	(6.0, 42.8)	63.2	(20.9, 100.0)	81.1	(49.9, 100.0)	82.2	(53.8, 100.0)	84.0	(58.7, 100.0)
4%												
Determine	4.3	(0.0, 9.1)	5.9	(1.1, 11.7)	4.9	(0.0, 11.0)	26.3	(4.7, 47.8)	45.3	(14.5, 76.2)	43.5	(14.3, 72.9)
Unigold	4.9	(0.0, 10.3)	20.4	(3.1, 37.6)	57.6	(14.3, 100.0)	77.3	(43.8, 100.0)	78.5	(48.0, 100.0)	80.6	(53.3, 100.0)
3%												
Determine	3.3	(0.0, 0.7)	4.5	(0.0, 9.5)	3.7	(0.0, 9.0)	20.9	(0.0, 40.8)	38.1	(7.9, 68.1)	36.4	(7.9, 64.8)
Unigold	3.7	(0.0, 8.4)	15.9	(0.0, 31.6)	50.2	(6.4, 94.0)	71.7	(35.6, 100.0)	73.0	(40.1, 100.0)	75.6	(45.8, 100.0)
2%												
Determine	2.1	(0.0, 5.6)	3.0	(0.0, 7.2)	2.5	(0.0, 6.8)	14.9	(0.0, 32.2)	28.9	(0.0, 56.9)	27.4	(1.1, 53.8)
Unigold	2.5	(0.0, 6.4)	11.1	(0.0, 24.6)	39.9	(0.0, 82.9)	62.5	(23.8, 100.0)	64.1	(28.6, 99.6)	67.1	(34.6, 00.7)
1%												
Determine	1.1	(0.0, 3.5)	1.5	(0.0, 4.5)	1.2	(0.0, 4.4)	7.9	(0.0, 21.2)	16.7	(0.0, 39.9)	15.7	(0.0, 37.3)
Unigold	1.2	(0.0, 4.0)	5.8	(0.0, 15.9)	24.7	(0.0, 62.6)	45.2	(5.4, 85.1)	46.9	(9.9, 83.9)	50.2	(15.6, 84.9)
Negative predictive value												
Incidence*	**3m**	**95% CI**	**6m**	**95% CI**	**9m**	**95% CI**	**12m**	**95% CI**	**15m**	**95% CI**	**18m**	**95% CI**
5%												
Determine	99.3	(97.2, 100.0)	99.8	(98.9, 100.0)	96.4	(91.1, 100.0)	99.9	(98.8, 100.0)	99.3	(94.2, 100.0)	99.9	(98.6, 100.0)
Unigold	99.7	(98.4, 100.0)	99.9	(98.9, 100.0)	98.7	(88.6, 100.0)	99.0	(91.2, 100.0)	99.4	(93.3, 100.0)	99.9	(98.3, 100.0)
4%												
Determine	99.4	(97.6, 100.0)	99.9	(99.0, 100.0)	97.1	(92.4, 100.0)	99.9	(98.9, 100.0)	99.5	(94.9, 100.0)	99.9	(98.7, 100.0)
Unigold	99.8	(98.6, 100.0)	99.9	(98.9, 100.0)	98.9	(89.9, 100.0)	99.2	(92.2, 100.0)	99.5	(94.1, 100.0)	99.9	(98.5, 100.0)
3%												
Determine	99.6	(97.9, 100.0)	99.9	(99.2, 100.0)	97.8	(93.8, 100.0)	99.9	(99.0, 100.0)	99.6	(95.7, 100.0)	99.9	(98.9, 100.0)
Unigold	99.8	(98.8, 100.0)	99.9	(99.1, 100.0)	99.2	(91.4, 100.0)	99.4	(93.4, 100.0)	99.6	(94.9, 100.0)	99.9	(98.7, 100.0)
2%												
Determine	99.7	(98.4, 100.0)	99.9	(99.3, 100.0)	98.6	(95.2, 100.0)	99.9	(99.2, 100.0)	99.7	(96.5, 100.0)	99.9	(99.1, 100.0)
Unigold	99.9	(99.1, 100.0)	99.9	(99.3, 100.0)	99.5	(93.2, 100.0)	99.6	(94.7, 100.0)	99.7	(95.6, 100.0)	99.9	(98.9, 100.0)
1%												
Determine	99.8	(98.9, 100)	99.9	(99.5, 100.0)	99.2	(96.9, 100.0)	99.9	(99.5, 100.0)	99.8	(94.2, 100.0)	99.9	(98.6, 100.0
Unigold	99.9	(99.4, 100.0)	99.9	(99.5, 100.0)	99.7	(95.3, 100.0)	99.8	(96.4, 100.0)	99.9	(97.2, 100.0)	99.9	(99.3, 100.0)

Abbreviations: NPV, negative predictive value; PPV, positive predictive value

*Incidence was defined as HIV infant incident infections occurring within the specified time period. For example, the PPV calculated at 6 months included infant infections

which occurred between 3 and 6 months.

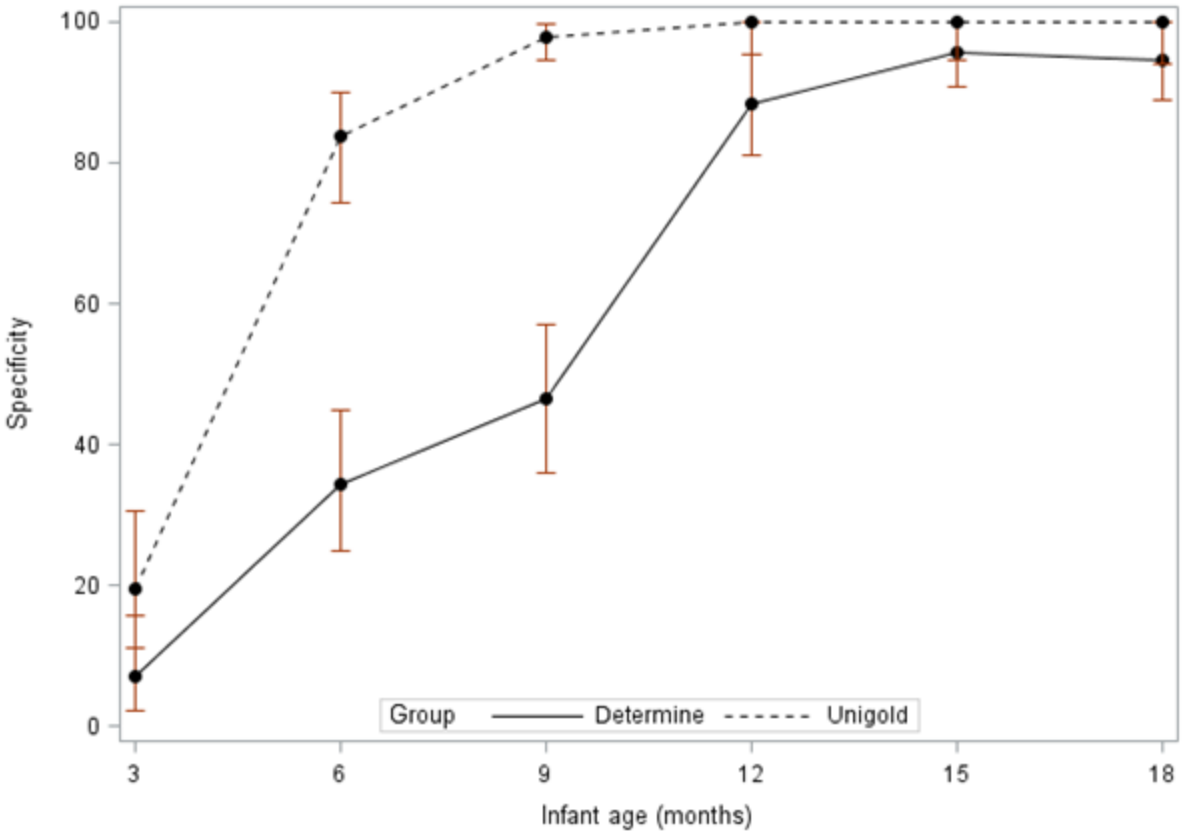
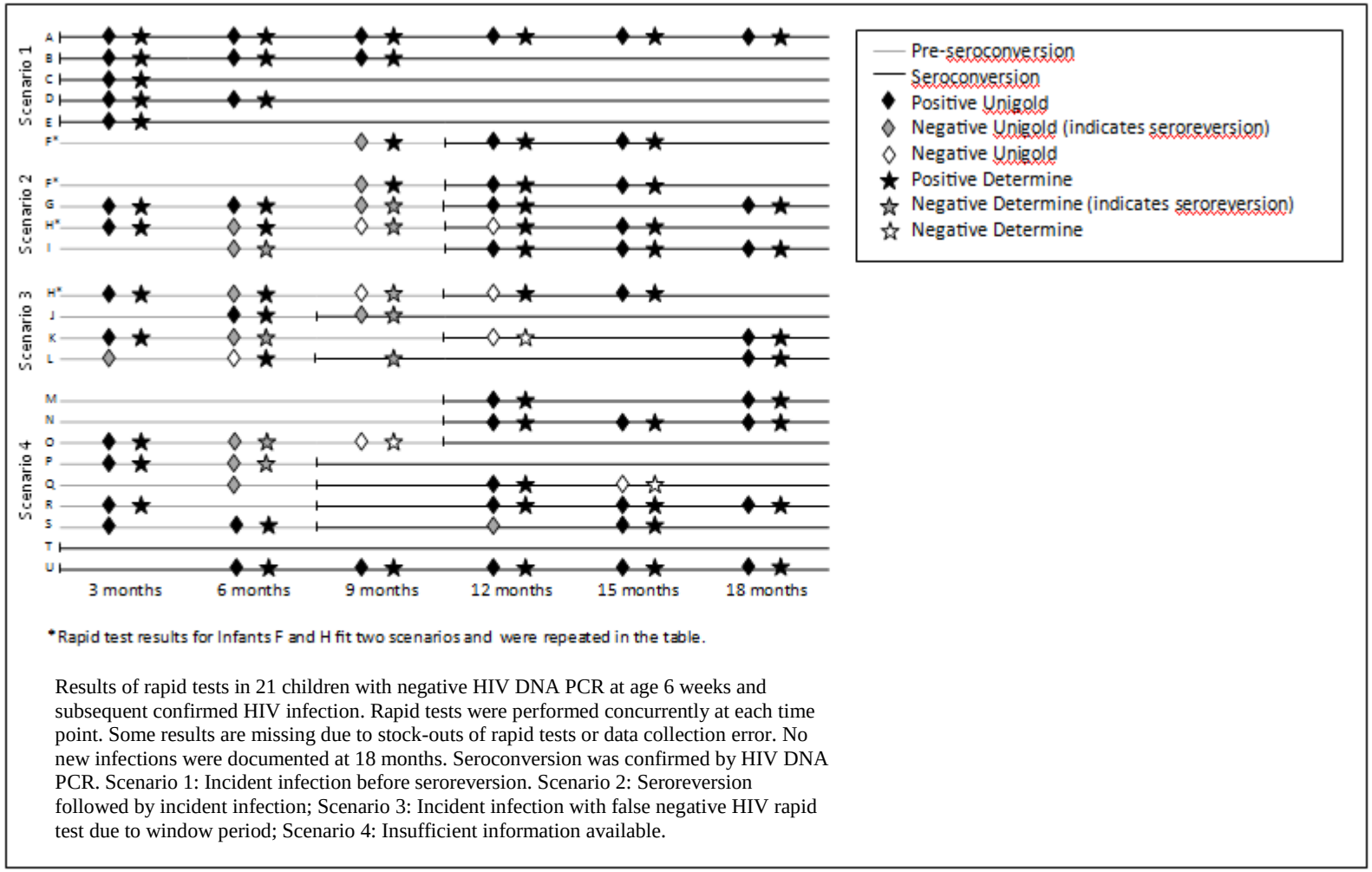


Figure 7. Specificity of Determine and Unigold assays during the postpartum period



*Rapid test results for Infants F and H fit two scenarios and were repeated in the table.

Results of rapid tests in 21 children with negative HIV DNA PCR at age 6 weeks and subsequent confirmed HIV infection. Rapid tests were performed concurrently at each time point. Some results are missing due to stock-outs of rapid tests or data collection error. No new infections were documented at 18 months. Seroconversion was confirmed by HIV DNA PCR. Scenario 1: Incident infection before seroreversion. Scenario 2: Seroreversion followed by incident infection; Scenario 3: Incident infection with false negative HIV rapid test due to window period; Scenario 4: Insufficient information available.

Figure 8. Results of rapid tests in 21 children with negative HIV DNA PCR at age 6 weeks and subsequent confirmed HIV infection

CHAPTER 6: RESULTS. TIMING OF HIV SEROREVERSION AMONG HIV-EXPOSED, BREASTFED INFANTS IN MALAWI: TYPE OF RAPID TEST MATTERS

The need for an accurate rapid HIV test for breastfeeding infants at point-of-care in resource-limited settings is now needed more than ever, given the recommendation for prolonged breastfeeding and increasing access to life-saving treatment for HIV-infected infants. Since 2010, the World Health Organization (WHO) breastfeeding guidelines[8] encourage women to exclusively breastfeed for the first six months of life and continue breastfeeding throughout the first two years of life, whereas before HIV positive mothers were encouraged to use formula feeding starting at age 6 months[182]. Results of studies led to a consensus that a prompt and definitive HIV diagnosis is crucial for timely initiation of life-saving antiretroviral treatment[9,183,184].

For early infant diagnosis (EID) of HIV infection, the WHO recommends a virological assay. Use of virological assays in resource-limited settings is limited by high costs and logistical constraints, as these assays require transportation to a centralized laboratory and thus a return visit by the mothers for test results. Rapid serological tests could be a cheaper, point-of-care alternative but they cannot distinguish between maternal and infant antibodies[13,14]. The lack of data on the timing of decay of maternal antibodies in young infants hinders the potential use of rapid tests for screening breastfeeding infants for mother to child transmission of HIV.

We aimed to determine the time to seroreversion for two commonly used rapid tests in a cohort of HIV-exposed breastfeeding infants age 3 to 18 months of life. A detailed description of the methods is available in section 4.2 of this document

6.1 Results

Of the 121 infants included in the analysis, 51% were female and 49% were male. The median age of the mother at the time of delivery was 27 years, most women were married (81%), only half completed the primary level of education or less. Most women self-reported no PMTCT for themselves (71%), and 80% of infants receiving single dose nevirapine or AZT, the regimens in use and available during the study period. Approximately 54% of infants were exclusively breastfed at month 3, only 18% were exclusively breastfed at month 6. Any breastfeeding, including mixed feeding, progressively declined with 87% of infants breastfeeding at 3 months, 69% at 6 months, 45% at 9 months, 39% at 12 months, 26% at 15 months, and 17% at 18 months of age. During follow-up, 21 infants were diagnosed with incident HIV infection. Twelve infants were censored due to an HIV infection or death occurring prior to seroreversion detected through Determine and 7 were censored due to an HIV infection or death occurring prior to seroreversion detected through Unigold.

The probability of seroreversion between age 3 and 18 months, stratified by rapid test, is presented in Table 15. At three months of age, 3% of infants had seroreverted according to Determine and 7% had seroreverted according to Unigold. About one in four infants had achieved seroreversion by 4 months using Unigold, but only by 6 months when using Determine. More than 95% of all infants had seroverted by 7 months according to Unigold and by 12 months according to the Determine assay.

6.2 Discussion

Circulating maternal HIV antibodies in HIV-exposed, uninfected infants complicates the interpretation of rapid serological tests due to the difficulty in distinguishing between a true positive (HIV antibodies produced by the HIV-infected infant) and a false positive (maternal HIV antibodies in the HIV-exposed, uninfected infant) result. In this cohort of HIV-exposed infants, we show that the time of seroreversion depends greatly on the type of test used, with time of seroreversion occurring at a much younger age for the Unigold assay compared to the Determine assay.

The WHO EID guidelines only recommend rapid tests for diagnostic purposes starting at age 18 months. Our data suggest that the Unigold assay could be used from month 7 onwards to exclude infant

HIV infection in HIV-exposed, breastfeeding infants, as maternal antibodies could no longer be detected by the Unigold assay in 98.9% (95% CI: 96.9, 100.0) of 7-month old infants. From a mother's perspective, an HIV negative diagnosis in her infant is emotionally important and could motivate her to adhere to her antiretroviral therapy during the remaining breastfeeding period. For healthcare providers, the documented time of seroreversion could increase confidence in screening for incident infant infections during the subsequent months of breastfeeding, as a positive rapid test after documented seroreversion will have a high positive predictive value and a negative test will have a high negative predictive value.

While data are limited, data on time to seroreversion greatly differs between different rapid tests, but also between studies using the same rapid test. Studies in industrialized countries using Western Blot assays reported median ages at seroreversion of 7 to 12 months[107-109]. A cross-sectional study of 12-month old South African infants documented seroreversion in 90% for Unigold and 55% for Determine[73], lower than the 99% for Unigold and 95% for Determine we observed at age 12 months. In another cross-sectional South African study, the proportion of HIV-exposed uninfected infants who seroreverted according to Determine was 20% at 4 months of age, 50% at 6 months, and 100% at 8 months of age[17], higher than the 8%, 25%, and 72% we observed in our cohort at the respective ages. Reasons for the marked differences between studies may be attributable to differences in postmenstrual age at delivery, maternal HIV immunoglobulin G (IgG) concentrations, maternal HIV severity, and maternal viral load and CD4 count, as these factors are strong determinants of the amount of placental immunoglobulin G (IgG) transfer[85-88 105,106].

In conclusion, our results highlight the need for recommendations to specify the timing and type of test used in the context of infant HIV detection in resource-poor settings, and base the interpretation of test result on knowledge of time to seroreversion of the selected test. Further research is needed in different resource poor settings to estimate the time of seroreversion, identify the optimal rapid test for repeat screening of breastfeeding infants, and better understand the determinants of seroreversion in this population.

Table 15. Estimated probability of seroreversion at a certain age, by rapid test

Age (month)	**Determine** Probability (95% CI)	**Unigold** Probability (95% CI)
3	3.1 (0.0, 6.7)	7.4 (1.0, 13.7)
4	8.1 (1.0, 14.5)	23.9 (14.5, 33.4)
5	16.5 (9.1, 23.9)	33.3 (30.0, 36.4)
6	24.9 (17.6, 32.3)	67.3 (61.7, 72.9)
7	72.4 (71.3, 73.4)	98.9 (96.9, 100.0)
8	72.4 (71.3, 73.4)	99.2 (97.1, 100.0)
9	85.5 (82.3, 88.7)	99.3 (97.3, 100.0)
10	90.4 (87.3, 93.6)	99.4 (97.4, 100.0)
11	92.3 (89.4, 95.1)	99.5 (97.6 (100.0)
12	95.1 (92.2, 97.9)	99.7 (97.7, 100.0)

*CI-confidence interval

CHAPTER 7: EVALUATION OF POINT-OF-CARE EARLY INFANT HIV DIAGNOSIS ALGORITHMS AMONG HIV-EXPOSED INFANTS DURING THE BREASTFEEDING PERIOD: A COST-EFFECTIVENESS ANALYSIS

Recent changes in the World Health Organization (WHO) breastfeeding guidelines will likely shift the majority of new infant HIV infections to the postnatal period. Based on research findings that exclusive breastfeeding improves HIV free survival and decreased HIV transmission in exposed infants[44-52,54-56], the WHO now encourages all HIV positive mothers to exclusively breastfeed for six months and then continue breastfeeding throughout the first or second year of the infant's life[2]. As this extends the duration of HIV exposure, it is estimated that 8 out of 10 new pediatric HIV infections will occur during the postpartum period[1].

Timely and accurate early infant diagnosis (EID) testing among HIV-exposed infants is critical during the first two years of life as disease progression occurs rapidly in young children[9,25,26]. EID testing recommendations have not yet been updated to incorporate the extended exposure risk period following implementation of the revised breastfeeding guidelines. Current EID guidelines recommend the use of virological assays[10] but their use is limited by financial and logistical constraints in resource limited settings[21,24]. Serological rapid tests are a cheaper, point-of-care (POC) alternative but cannot distinguish between maternal and infant antibodies at young ages and perform differently depending on the type of rapid test[13,14]. Qualitative virological POC tests are being developed[185,186], but they will most likely be much more expensive than serological rapid tests.

Using Markov models, we aimed to assess the clinical and economic outcomes, and cost-effectiveness of different POC-based EID strategies that can be incorporated within existing PMTCT structures and vaccination schedules.

7.1 Base case results (see Appendix B, Table 20 for full results)

Economic outcomes

Table 16 outlines the total costs of the 21 EID testing strategies. Testing only once with Unigold were the three least expensive options, at $18.20, $23.37, and $30.62 per HIV-exposed infant for testing at 9, 12 and 6 months, respectively. Testing twice with Alere were the three most expensive strategies, at $145.60, $142.70, and $131.00 per HIV-exposed infant for testing at 6 and 15 months, 6 and 12 months, and 9 and 18 months, respectively. For each of the time points assessed (i.e., WHO recommended time points, testing only once, and testing twice) Unigold was the least expensive and Alere the most expensive.

Clinical outcomes

The strategy with the lowest effectiveness, measured as the highest accumulated DALYs, was testing only once at 9 months with Unigold (337,806), followed by testing at 9 months with Determine (327,377) or testing at 6 months with Unigold (310,881) (Table 16). The strategy with the greatest effectiveness was testing twice at 6 and 15 months with Alere at 6 and 15 months (192,588), followed by testing at 9 and 18 months or 6 and 12 months with Alere (197,025 and 198,132, respectively).

Cost-effectiveness

All EID strategies were ranked in sequential order by total costs, from lowest to highest total costs and the effectiveness of each alternative strategy was compared with the next more expensive strategy in terms of DALYs averted. After eliminating dominated strategies with higher DALYs in the next more expensive strategy, four alternative strategies remaining cost-effective (Table 17 and Figure 9). Testing only once at 12 months with Unigold had an incremental cost-effectiveness of an additional $1.53 per DALY averted as compared to testing only once at 9 months with Unigold. Testing only once at 12 months with Determine had an ICER of $3.94 per DALY averted as compared to testing only once at 12 months with Unigold. Testing only once at 15 months with Determine had an ICER of $3.02 per DALY averted as compared to testing only once at 12 months with Determine. Testing only once at 15 months with Alere had an ICER of $15.20 per DALY averted as compared to testing only once at 15 months with

Determine. Testing twice at 6 and 15 months with Unigold had an ICER of $6.37 per DALY averted as compared to testing only once at 15 months with Alere. Lastly, testing twice at 6 and 15 months with Alere had an ICER of $91.98 per DALY averted as compared with testing twice at 6 and 15 months with Unigold. All six undominated strategies were lower than the willingness-to-pay threshold of the per capita Malawi GDP value of $275.00[187].

7.2 Discussion

Cost-effective EID strategies are needed to reduce the burden of pediatric HIV in sub-Saharan Africa, particularly given the predicted relative increase in HIV infections due to breastfeeding following the recent WHO recommendation that all HIV positive women breastfeed their infant for at least 12 months. The current EID recommendations were made prior to these changes in breastfeeding guidelines. We assessed the cost-effectiveness of 21 EID strategies that varied in test type, number of testing timepoints, and timing of test. All strategies used POC tests in infants who tested HIV negative on a PCR test at age 4 to 6 weeks, in line with the WHO EID recommendations, and all strategies confirmed a positive POC test with a HIV DNA PCR assay or a HIV RNA viral load assay. Six strategies were deemed cost-effective: a single Unigold rapid test at 12 months was the most cost-effective strategy, followed by a single Determine test at 12 months, testing once at 15 months using Determine, a single POC qualitative virological Alere assay at 15 months, two Unigold tests at 6 and 15 months, or two Alere tests at 6 and 15 months. All six dominant EID strategies were highly cost-effective according to the commonly accepted WHO standards of <1 times or <3 times GDP per capita[187].

The factors contributing the most to the cost-effectiveness of EID strategies were the costs, performance of the assays evaluated, and testing timepoints. Although testing once at 9 months with Unigold was the lowest costing strategy, the DALYs were the highest. Conversely, the strategies with the lowest DALYs, or highest effectiveness, were some of the most expensive strategies (i.e. testing twice at 6 and 15 months with Alere or Unigold or testing twice at 9 and 18 months with Alere). The other factors significantly contributing to the costs of each strategy were the number of false positive and true positive test results. For each false positive result, a follow-up PCR, at an estimated $35 per test, and an extra pre-

and post-test counseling session was required. Since the number of false positive results was highest for Determine, these strategies had higher testing and counseling costs than Unigold or Alere. The number of true positive results increased healthcare and treatment costs. Consequently, most of the strategies using Alere had higher healthcare costs than strategies using Determine and Unigold.

Low effectiveness (i.e. high DALY's) was mostly due to missing HIV infections due to testing at early time points or due to low test sensitivity. Most of infections in our cohort occurred between 6 weeks and 3 months or after 6 or 9 months. Thus, strategies testing only once at time points before 12 months had higher numbers of missed infections and thus higher DALYs since we assumed these infections would be diagnosed at a later stage of disease. Furthermore, strategies with two testing strategies reduced the likelihood of a missed infection which, in turn, reduced overall DALYs. At any time point, lower test sensitivity resulted in a higher number of false negative results, and thus lower effectiveness. The highest number of false negative results occurred when testing only once at 9 months with Determine, which is not surprising as the Determine assay capture maternal HIV-antibodies at an older age than Unigold[179,180]. Strategies using the virological Alere assay had the lowest number of false positive test results, regardless of when testing occurred.

Our study was the first to evaluate the cost-effectiveness of EID strategies for several POC rapid tests in a cohort of predominantly breastfeeding infants in resource-limited settings. Thus, comparisons with other studies cannot be made. While there are more POC HIV tests commercially available and used in high burden countries, we only evaluated two serological rapid tests that are commonly used in the public sector in Malawi. We also evaluated a virological assay that is new and has not been extensively studied in resource-limited settings. However, the results from both studies examining the Alere were similar highlighting the strong performance in the field. The validity of any model greatly depends on the accuracy of parameter values used. Given the recent implementation of option B+ and the 2010 WHO guidelines, few prospective cohort data on infant outcomes under these policies is available. To refine the model and improve accuracy, data on postpartum maternal and infant ART adherence and other rapid tests data, including further studies with POC virological assays, should be incorporated once available.

Conclusion

The goal of any EID program is to both promptly identify pediatric incident HIV cases, thereby quickly initiating treatment to reduce HIV-related infant morbidity and mortality[9], and accurately rule out HIV infection in uninfected infants, thereby comforting women that their infant is free of HIV infection. In addition, EID programs need to be caregiver-friendly by limiting the number of times a caregiver has to return to the clinic. From a country-level standpoint, EID strategies need to be both cost-effective and affordable since the settings with the highest number of HIV-exposed infants are typically highly limited in their public health resources.

The current EID guidelines are outdated for the new WHO breastfeeding guidelines, which increase the duration infants born to HIV positive mothers are exposed to HIV. Furthermore, even though it has been demonstrated that different assays perform differently in young infants[17,73-75], the guidelines do not aid countries in determining which type of test should be used in their EID program. Our analyses show that the type of test and timing of test matters greatly to the cost-effectiveness of EID strategies, with the lowest costing cost-effective strategy being a single Unigold rapid test at age 12 months. Among the six cost-effective strategies, testing twice at 6 and 15 months with Alere was the most effective but also the most expensive. For maximum impact from an EID strategy in terms of the lowest DALYs, a low-cost qualitative virological assay at POC would be the ideal assay during the breastfeeding period in high burden countries.

Table 16. Base case results: POC test results, total costs, and pediatric outcomes for early infant diagnosis algorithms
(See Appendix B: Table 20 for complete results)

EID algorithm	Testing month	Test type	Economic outcomes				
			Testing	Counseling	Healthcare / treatment	Total Costs	Costs per infant
Current WHO recomme-ndations	**9, 18**	DT	$98,337.53	$12,130.56	$568,126.05	$1,031,287.06	$103.13
		UN	$94,149.19	$12,818.95	$666,698.70	$818,084.13	$81.81
		Alere	$266,774.51	$10,836.54	$449,417.75	$1,310,070.88	$131.00
EID strategies with 1 testing timpeoint	**6**	DT	$220,839.44	$18,052.14	$231,283.92	$470,175.50	$47.02
		UN	$93,030.41	$11,364.86	$201,760.81	$306,156.08	$30.62
		Alere	$249,780.17	$10,075.04	$223,104.99	$482,960.20	$48.30
	9	DT	$179,615.78	$16,109.78	$121,062.78	$316,788.34	$31.68
		UN	$56,904.26	$10,164.89	$115,514.89	$182,584.04	$18.20
		Alere	$259,293.25	$10,448.69	$204,539.60	$474,281.54	$47.43
	12	DT	$69,379.84	$11,860.47	$252,520.93	$333,761.24	$33.38
		UN	$56,346.84	$10,158.83	$167,187.68	$233,693.35	$23.37
		Alere	$249,681.72	$10,076.25	$237,820.59	$497,578.57	$49.76
	15	DT	$55,716.61	$11,939.74	$318,324.43	$385,980.78	$38.60
		UN	$69,730.75	$11,509.08	$305,966.19	$387,206.01	$38.72
		Alere	$291,582.00	$11,801.72	$373,726.37	$677,110.09	$67.71
EID strategies with 2 testing timepoint s	**6, 12**	DT	$123,627.85	$14,749.07	$530,144.61	$1,197,966.48	$119.80
		UN	$111,432.50	$13,390.03	$469,544.76	$969,934.18	$96.99
		Alere	$307,975.62	$12,509.76	$514,047.55	$1,427,732.29	$142.70
	6, 15	DT	$113,145.90	$13,944.13	$613,104.75	$1,269,639.73	$126.96
		UN	$113,746.67	$12,978.91	$510,876.32	$1,013,168.78	$101.32
		Alere	$318,243.18	$12,927.28	$532,419.63	$1,456,789.45	$145.60

*DALY-disability-adjusted life years; DT-Determine; FN-false negative; FP-false positive; TN-true negative; TP-true positive; UN-Unigold4

Note: Costs were ranked from low to high by total costs of each EID strategy. DALYs were ranked from low to high by total DALYs for each EID strategy. Costs ranking were used in sequential comparisons to compute the ICERs. DALY rankings are presented here to describe the base case results and were not used for sequential comparisons for ICER calculations.

Table 16 (continued). Base case results: POC test results, total costs, and pediatric outcomes for early infant diagnosis algorithms (See Appendix B: Table 20 for complete results)

			Clinical outcomes						Rank (low to high)	
EID algorithm	**Testing month**	**Test type**	**TP**	**FN**	**FP**	**TN**	**Missed infections**	**DALY**	**Cost**	**DALY**
Current WHO recommend-ations	**9, 18**	DT	1,046	0	163	6,084	584	273,521	18	13
		UN	1,228	0	0	6,525	503	252,403	15	11
		Alere	828	0	0	5,967	473	197,025	19	2
EID strategies with 1 testing timepoint	**6**	DT	426	0	5,286	1,031	1,293	297,705	8	15
		UN	372	0	1,047	5,152	1,402	310,881	3	19
		Alere	411	5	16	6,277	1,353	307,921	10	18
	9	DT	223	257	4,322	1,818	1,321	327,377	6	20
		UN	213	142	213	5,798	1,507	337,806	1	21
		Alere	377	4	16	6,619	1,190	273,279	9	12
	12	DT	465	0	899	5,612	1,163	278,726	4	14
		UN	308	243	0	6,353	1,151	304,094	2	17
		Alere	438	4	14	6,226	1,313	303,634	11	16
	15	DT	586	147	342	6,417	733	243,559	7	9
		UN	564	141	0	6,872	704	233,526	5	6
		Alere	688	1	8	6,938	689	219,955	12	5
EID strategies with 2 testing timepoints	**6, 12**	DT	976	0	919	6,540	462	237,607	16	8
		UN	865	159	0	7,552	483	250,767	13	10
		Alere	947	11	14	6,867	459	234,281	20	7
	6, 15	DT	1,129	97	338	6,742	160	217,992	17	4
		UN	941	98	0	7,156	163	198,132	14	3
		Alere	981	4	14	7,099	228	192,588	21	1

*DALY-disability-adjusted life years; DT-Determine; FN-false negative; FP-false positive; TN-true negative; TP-true positive; UN-Unigold4

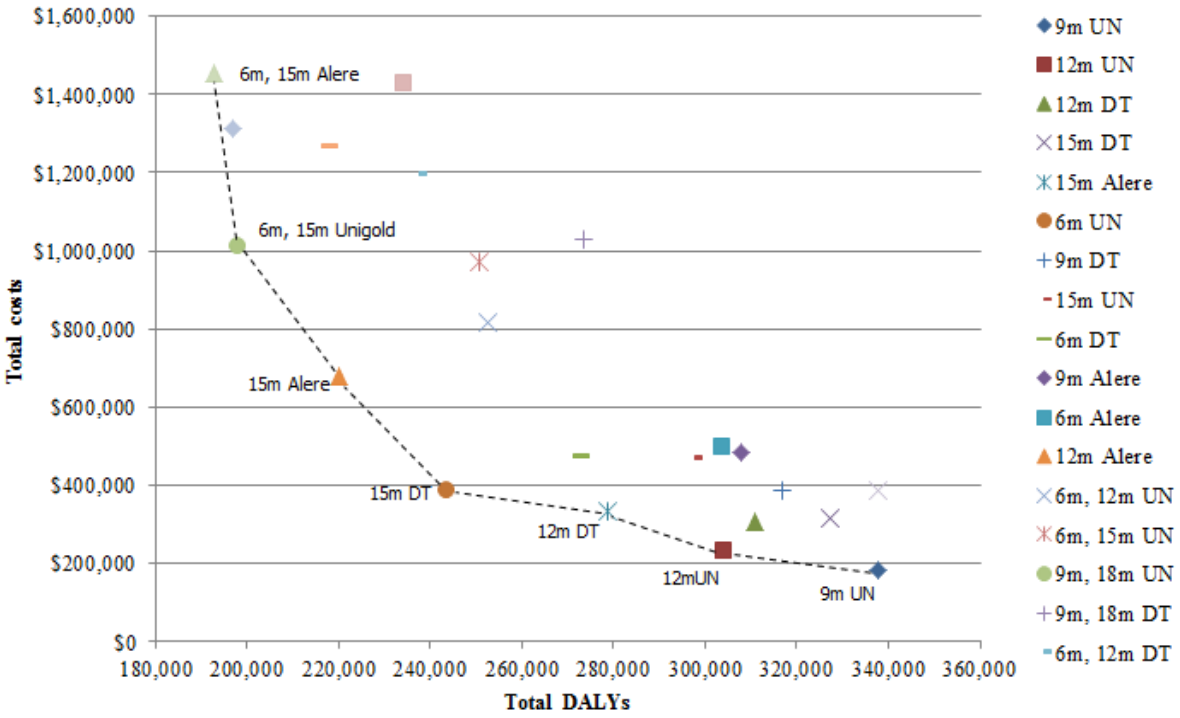
Note: Costs were ranked from low to high by total costs of each EID strategy. DALYs were ranked from low to high by total DALYs for each EID strategy. Costs ranking were used in sequential comparisons to compute the ICERs. DALY rankings are presented here to describe the base case results and were not used for sequential comparisons for ICER calculations.

Table 17. Incremental cost-effectiveness ratio (ICER) comparison of EID strategies ranked by total EID costs

EID strategy	EID strategy cost	EID strategy DALY	Incremental cost	Incremental DALY averted	ICER
9m UN	$182,140.26	337,806.02	-	-	-
12m UN	$233,724.16	304,093.94	$51,703.28	33,712.08	$1.53
6m UN	$306,364.94	310,880.97	$72,462.73	(6,787.03)	Dominated
8m DT	$316,775.96	327,376.78	$10,632.26	(16,495.81)	Dominated
12m DT	$333,682.30	278,726.36	$16,972.90	48,650.42	$3.94
15m DT	$385,795.72	243,559.47	$52,219.54	35,166.89	$3.02
15m UN	$387,206.01	316,942.63	$1,225.23	(73,383.16)	Dominated
6m DT	$470,158.02	297,645.18	$82,696.12	19,297.44	Dominated
9m Alere	$474,445.44	273,278.99	$4,379.42	24,366.19	Dominated
6m Alere	$482,974.32	307,920.81	$8,678.65	(34,641.81)	Dominated
12m Alere	$497,565.56	303,633.79	$14,618.37	4,287.01	Dominated
15m Alere	$676,912.75	219,954.99	$179,531.53	83,678.80	$15.20
6m, 12m UN	$918,166.50	252,402.55	$140,974.04	(32,447.56)	Dominated
6m, 15m UN	$950,461.22	250,766.65	$151,850.05	1,635.90	$6.37
9m, 18m UN	$1,063,564.22	198,131.88	$43,234.61	52,634.78	Dominated
9m, 18m DT	$1,135,204.22	273,521.20	$18,118.28	(75,389.32)	Dominated
6m, 12m DT	$1,152,777.98	237,606.58	$166,679.42	35,914.62	Dominated
6m, 15m DT	$1,191,265.56	217,991.71	$71,673.24	19,614.87	Dominated
9m, 18m Alere	$1,290,269.64	197,024.92	$42,777.34	20,966.79	Dominated
6m, 12m Alere	$1,429,630.58	234,281.34	$114,578.70	(37,256.42)	Dominated
6m, 15m Alere	$1,458,109.54	192,588.02	$29,046.50	41,693.32	$91.98

*DT-Determine; EID-early infant diagnosis; UN-Unigold

Note: We assessed the relative value of each EID strategy to the next most expensive strategy using the incremental cost-effectiveness ratio (ICER) by ranking all EID strategies in sequential order by total costs. The effectiveness of each EID strategy was evaluated as DALYs averted compared with the next most expensive strategy. Strategies were considered dominated if DALYs were not averted in the next most expensive strategy. After the initial comparisons of all strategies in sequential order, dominated strategies were eliminated from the cost rankings and the effectiveness of the remaining strategies were evaluated again as DALYs averted, compared with the next most expensive strategy. This iterative process continued until all remaining strategies were undominated by the next most expensive strategy, resulting in the final ICER calculations.



*DT-Determine; EID-early infant diagnosis; UN-Unigold

Note: We assessed the relative value of each EID strategy to the next most expensive strategy using the incremental cost-effectiveness ratio (ICER) by ranking all EID strategies in sequential order by total costs. The effectiveness of each EID strategy was evaluated as DALYs averted compared with the next most expensive strategy. Strategies were considered dominated if DALYs were not averted in the next most expensive strategy. Dominant strategies are labeled and are connected with the dotted line. All dominant strategies tested only once at the specified timepoint with specified POC test.

Figure 9. Cost-effectiveness efficiency frontier

CHAPTER 8: CONCLUSION

The current PMTCT cascade has resulted in dramatic reductions in pediatric HIV infections, particularly during the prenatal or delivery period[1,19,22], but guidelines and PMTCT activities are lacking for the postpartum period[4]. Recent changes in the WHO breastfeeding guidelines will increase the HIV exposure period for infants since all HIV positive women are now encouraged to breastfeed for the first one to two years of the infant's life[2]. It is estimated that, in breastfeeding populations, up to 80% of all new infections will occur in the postpartum period[1]. Updated PMTCT and early infant diagnosis (EID) guidelines are thus needed in light of the extended breastfeeding period to ensure prompt identification of infant infection and initiation of life-saving antiretroviral treatment.

Although ART can significantly reduce the risk of transmission associated with mixed feeding[57-62], ART adherence during the postpartum period is generally lower than during the prenatal period, especially in women who are first diagnosed with HIV during their pregnancy[147,188-190]. Poor adherence to ART in the postpartum period is thus likely to remain a problem, even in the Option B+ era .[147,149,191] EID after 9 or 12 months of life will thus remain critical to optimal care for HIV-exposed infants.

Type of test used for EID matters

Serological rapid tests are a cheaper alternative than virological tests and eliminate the need for a breastfeeding mother to return to the clinic for results. The WHO recommendations include using a rapid test but only for diagnosis of HIV infection after age 18 months or to determine if a child has been exposed to HIV. Furthermore, the guidelines do not provide guidance on which one to use[10].

Our data showed that rapid tests perform differently depending on the type and timing of the test.

Starting at age 6 months, the Unigold test could be used as a screening tool in the follow-up of HIV-exposed infants, which is earlier than the current recommended time of 9 months of age. Additionally, we showed the type of rapid test matters and differs greatly in regards to sensitivity and specificity particularly during the early infancy months. Unigold outperformed Determine in regard to specificity at all timepoints, thus reducing the likelihood of a mother receiving a false positive result. This may encourage the mother to adhere to treatment while she continues breastfeeding her HIV uninfected infant. Strictly looking only at specificity, our data suggests using Unigold for EID testing, especially prior to 12 months of age. However, specificity is only one part of what matters for EID.

In regards to sensitivity, both rapid tests missed pediatric HIV infections beginning at 9 months of age. Since is it critical to timely identify an incident infection as disease progression occurs rapidly in young infants, these missed infections are associated with poorer prognosis and higher mortality than infections accurately identified[25,26]. New virological assays for use at point of care that are highly sensitive and specific are being developed but will be significantly more expensive than serological rapid tests[185,186].

Timing and type of test greatly determine the cost-effectiveness of EID strategies

The current WHO recommendations include testing at 9 and 18 months of age or 6 weeks after breastfeeding cessation[27]. Our data suggests that testing at 9 months may be too early or too late to begin EID testing since most infections occur prior to 3 months or after 9 months. The higher rate of infections after 9 months may be due to the switch from exclusive breastfeeding to mixed feeding at age 6 months, as mixed feeding is known to increase the risk of HIV transmission to her infant through breastfeeding[46,55,56,192]. Other studies, including two trials of ART prophylaxis in the first two years of life among Malawian infants and a meta-analysis among HIV-exposed infants in multiple African countries, similarly found that the risk of postpartum, breastfeeding-associated HIV transmission was greatest within the first 6 weeks of life and after 9 months of age[133,134]. Testing at later months (i.e., 12 and 15 months) in our data therefore resulted in reductions in missed infections and increases in DALYs,

regardless of type of rapid test. Among the 21 EID strategies evaluated, four cost-effective strategies with single testing timepoints implemented testing after age 9 months. The remaining two cost-effective strategies with two testing timepoints tested early at 6 months, which would have timely diagnosed infections prior to 3 months, and again after 9 months, which would have diagnosed later infections occurring after 9 months.

Our data suggests that a low-cost POC virological assay could become the most attractive EID strategy for high burden countries. Until such tests are available, four cost-effective EID strategies using rapid tests that are more cost-effective than the current EID guidelines could already be implemented: testing once at 12 months with Unigold or Determine, testing once at 15 months with Determine, and testing twice at 6 and 15 months with Unigold.

Redefining the PMTCT cascade in the option B+ era: a mother-infant dyad approach to incorporate the postpartum period

Even though the simplified treatment approaches of Option B and Option B+ have significantly increased the number of HIV positive, pregnant women *initiating* ART, significant challenges remain. Only 4 of the 22 priority countries identified in the Global Plan achieved the goal of providing ART to 90% of pregnant women living with HIV. Among women residing in sub-Saharan Africa, only 59% received ART therapy or prophylaxis during pregnancy and delivery in 2011[19,63]. Additionally, only 58% of mothers[64] and 29-41% of HIV-exposed infants received ART therapy in 2009[65,66]. Thus, EID will remain a key component of the PMTCT cascade for the near future unless innovative approaches significantly improve ART adherence.

Even among mothers with access to Option B+, attrition in the postpartum period has been worse than in the prenatal period, in part due to PMTCT scale-up being focused towards pregnant women, frequent maternal HIV non-disclosure, and lack of care engagement[67,193]. The highest rates of treatment default occurs after 6 weeks postpartum and increases progressively throughout the first 12 months[11,31,36,37,42,68,69]. These missed opportunities affect both the health of the breastfeeding HIV positive mother and their infant. If mothers do not return for care, their infants are also not returning for EID.

Thus, the PMTCT cascade should be focused on the mother-infant dyad and include clear guidelines for EID testing and linkage and engagement in care among breastfeeding, HIV positive mothers,

Conclusion

It is critical to advocate for an EID testing regimen that is mother-friendly, by being at the point-of-care, and accurate, by reducing the number of false positive and false negative results. EID strategies that are more likely to result in a true negative or a true positive result will give a breastfeeding mother peace-of-mind about her infant's HIV exposure and encourage continued adherence to her ART regimen until she ceases breastfeeding, which in turn will reduce the infant's risk of HIV transmission. From a country's perspective, high burden settings are often tasked with weighing the benefits of a specific intervention with the costs of implementation. Our data helps guide countries toward cost-effective, mother-friendly EID strategies from both the individual (i.e., HIV positive mother and her breastfeeding infant) and population (i.e., high burden setting) perspective.

APPENDIX A. COMPARISON BETWEEN DEMOGRAPHIC CHARACTERISTICS IN COMPLETE CASE VERSUS MISSING POPULATION

Table 18. Comparison of demographic characteristics in complete case population versus missing population: Determine

	3 months			6 month			9 month		
	CC	Missing		CC	Missing		CC	Missing	
	N (%)	N (%)	p-value	N (%)	N (%)	p-value	N (%)	N (%)	p-value
Infant characteristics									
Gender									
Female	37 (49)	24 (53)		48 (49)	14 (54)		44 (48)	18 (60)	
Male	39 (51)	21 (47)	0.62	49 (51)	12 (46)	0.69	48 (52)	12 (40)	0.25
EBF									
Yes	42 (55)	23 (51)		19 (20)	3 (12)		1 (1)	0 (0)	
No	27 (36)	16 (36)	0.52	68 (70)	16 (61)	0.05	83 (90)	16 (53)	<0.0001
Missing	7 (9)	6 (13)	0.85	10 (10)	7 (27)	0.56	8 (9)	14 (47)	0.66
ABF									
Yes	67 (88)	38 (85)		70 (72)	15 (58)		46 (50)	9 (30)	
No	2 (3)	1 (2)	0.51	21 (22)	5 (19)	0.03	42 (46)	8 (27)	<0.0001
Missing	7 (9)	6 (13)	0.92	6 (6)	6 (23)	0.85	4 (4)	13 (43)	0.96
NVP at birth									
No	4 (5)	2 (4)		6 (6)	1 (4)		6 (7)	0 (0)	
Yes	56 (74)	40 (89)	0.10	75 (77)	22 (85)	0.79	70 (76)	27 (90)	0.93
Missing	16(21)	3 (7)	0.69	16 (16)	3 (11)	0.61	16 (17)	3 (10)	0.15
Maternal characteristics									
Age (mean)	28	29	0.22	28	29	0.23	28	29	0.23
Maternal ARV prior to birth									
Yes	50 (66)	35 (78)		70 (72)	17 (65)		62 (67)	24 (80)	
No	17 (23)	6 (13)	0.24	17 (18)	6 (23)	0.59	20 (22)	3 (10)	0.34
Missing	9 (11)	4 (9)	0.20	10 (10)	3 (12)	0.49	10 (11)	3 (10)	0.15
Marital status									
Single	63 (88)	38 (88)		80 (87)	23 (92)		76 (87)	26 (89)	
Married	9 (12)	5 (12)	0.89	12 (13)	2 (8)	0.49	11 (13)	3 (11)	0.74
Education level									
≥ secondary	15 (21)	11 (26)		19 (21)	7 (28)		20 (23)	6 (21)	
< secondary	58 (79)	32 (74)	0.56	73 (79)	18 (72)	0.44	67 (77)	23 (79)	0.79
Access to water in home									
Yes	51 (73)	28 (67)		63 (71)	17 (71)		62 (73)	18 (64)	
No	19 (27)	14 (33)	0.49	26 (29)	7 (29)	0.99	23 (27)	10 (36)	0.38
Electricity in home									
Yes	49 (68)	25 (58)		63 (68)	13 (52)		59 (68)	16 (55)	
No	23 (32)	18 (42)	0.28	29 (32)	12 (48)	0.13	28 (32)	13 (45)	0.22
Building material of roof									
Iron/tiles	63 (89)	36 (84)		76 (84)	24 (96)		72 (84)	28 (96)	
Grass	8 (11)	7 (16)	0.45	15 (16)	1 (4)	0.11	14 (16)	1 (4)	0.08

**CC: Complete case population; EBF: Exclusive breastfeeding; ABF: Any breastfeeding

Table 18 (continued). Comparison of demographic characteristics in complete case population versus missing population: Determine

	12 month			15 month			18 month		
	CC	**Missing**		**CC**	**Missing**		**CC**	**Missing**	
	N (%)	**N (%)**	**p-value**	**N (%)**	**N (%)**	**p-value**	**N (%)**	**N (%)**	**p-value**
Infant characteristics									
Gender									
Female	44 (51)	19 (53)		43 (57)	18 (40)		35 (55)	31 (48)	
Male	43 (49)	17 (47)	0.83	33 (43)	27 (60)	0.08	29 (45)	34 (52)	0.43
EBF									
Yes	1 (1)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
No	80 (92)	16 (44)	<0.0001	68 (90)	17 (38)	<0.0001	60 (94)	28 (43)	<0.0001
Missing	6 (7)	20 (56)	0.66	8 (10)	28 (62)	-	4 (6)	37 (57)	-
ABF									
Yes	39 (45)	8 (22)		23 (30)	9 (20)		15 (23)	8 (12)	
No	42 (48)	8 (22)	<0.0001	45 (59)	8 (18)	<0.0001	47 (74)	21 (32)	<0.0001
Missing	6 (7)	20 (56)	0.89	8 (11)	28 (62)	0.15	2 (3)	36 (56)	0.73
NVP at birth									
No	5 (6)	1 (3)		4 (5)	2 (4)		3 (5)	5 (8)	
Yes	69 (79)	28 (78)	0.39	64 (84)	32 (71)	0.08	52 (81)	48 (74)	0.86
Missing	13 (15)	7 (19)	0.52	8 (11)	11 (25)	1.00	9 (14)	12 (18)	0.53
Maternal characteristics									
Age (mean)	28	29	0.23	28	29	0.23	28	29	0.23
Maternal ARV prior to birth									
Yes	61 (70)	26 (72)		53 (69)	32 (71)		53 (83)	40 (62)	
No	18 (21)	5 (14)	0.85	17 (22)	6 (13)	0.62	9 (14)	14 (22)	0.003
Missing	8 (9)	5 (14)	0.44	6 (8)	7 (16)	0.31	2 (3)	11 (17)	0.13
Marital status									
Single	73 (89)	30 (86)		62 (86)	39 (91)		54 (87)	55 (90)	
Married	9 (11)	5 (14)	0.62	10 (14)	4 (9)	0.49	8 (13)	6 (10)	0.59
Education level									
≥ secondary	17 (21)	9 (26)		17 (24)	9 (21)		13 (21)	13 (21)	
< secondary	65 (79)	26 (74)	0.55	55 (76)	34 (79)	0.74	49 (79)	48 (79)	0.96
Access to water in home									
Yes	59 (75)	21 (60)		51 (73)	28 (67)		43 (73)	42 (71)	
No	20 (25)	14 (40)	0.12	19 (27)	14 (33)	0.49	16 (27)	17 (29)	0.84
Electricity in home									
Yes	54 (66)	22 (63)		47 (65)	27 (63)		40 (65)	42 (69)	
No	28 (34)	13 (37)	0.76	25 (35)	16 (37)	0.79	22 (35)	19 (31)	0.61
Building material of roof									
Iron/tiles	67 (83)	34 (97)		59 (83)	40 (93)		52 (84)	53 (88)	
Grass	14 (17)	1 (3)	0.03	12 (17)	3 (7)	0.13	10 (16)	7 (12)	0.48

**CC: Complete case population; EBF: Exclusive breastfeeding; ABF: Any breastfeeding

Table 19. Comparison of demographic characteristics in complete case population versus missing population: Unigold

	3 months			6 month			9 month		
	CC	**Missing**		**CC**	**Missing**		**CC**	**Missing**	
	N (%)	**N (%)**	**p-value**	**N (%)**	**N (%)**	**p-value**	**N (%)**	**N (%)**	**p-value**
Infant characteristics									
Gender									
Female	38 (49)	23 (52)							
Male	39 (51)	21 (48)	0.76			0.32			0.35
EBF									
Yes				19 (18)	3 (17)		1 (1)	0 (0)	
No			0.28	76 (72)	8 (44)	0.03	81 (89)	18 (58)	<0.0001
Missing			0.44	10 (10)	7 (29)	0.56	9 (10)	13 (42)	0.64
ABF									
Yes				77 (73)	8 (45)		45 (49)	10 (32)	
No			0.46	22 (21)	4 (22)	0.0008	41 (45)	9 (29)	0.0006
Missing			0.95	6 (6)	6 (33)	0.38	5 (6)	12 (39)	0.98
NVP at birth									
No	4 (5)	2 (5)							
Yes	57 (74)	39 (89)	0.11			0.48			0.54
Missing	16 (21)	3 (7)	0.73			0.26			0.12
Maternal characteristics									
Age (mean)	28	29	0.32	28	29	0.32	28	29	0.32
Maternal ARV prior to birth									
Yes							60 (66)	26 (84)	
No			0.82			0.49	20 (22)	3 (10)	0.09
Missing			0.82			0.93	11 (12)	2 (6)	0.10
Marital status									
Single									
Married			0.51			0.41			0.69
Education level									
≥ secondary									
< secondary			0.25			0.44			0.89
Access to water in home									
Yes									
No			0.97			0.85			0.80
Electricity in home									
Yes				8 (47)	68 (68)				
No			0.41	9 (53)	32 (32)	0.10			0.29
Roof									
Iron/tiles							29 (97)	71 (84)	
Grass			0.79			0.31	1 (3)	14 (16)	0.07

**CC: Complete case population; EBF: Exclusive breastfeeding; ABF: Any breastfeeding

Table 19 (continued). Comparison of demographic characteristics in complete case population versus missing population: Unigold

	12 month			15 month			18 month		
	CC	Missing		CC	Missing		CC	Missing	
	N (%)	N (%)	p-value	N (%)	N (%)	p-value	N (%)	N (%)	p-value
Infant characteristics									
Gender									
Female				44 (60)	17 (35)				
Male			0.66	29 (40)	31 (65)	0.01			0.44
EBF									
Yes	1 (1)	0 (0)		0(0)	0 (0)		0 (0)	0 (0)	
No	83 (92)	13 (39)	<0.0001	64 (88)	21 (44)	<0.0001	64 (94)	24 (39)	<0.0001
Missing	6 (7)	20 (61)	0.69	9 (12)	27 (56)	-	4 (6)	37 (61)	-
ABF									
Yes	42 (47)	5 (15)		23 (32)	9 (19)		17 (25)	6 (10)	
No	42 (47)	8 (24)	<0.0001	41 (56)	12 (25)	<0.0001	49 (72)	19 (31)	<0.0001
Missing	6 (6)	20 (61)	0.44	9 (12)	27 (56)	0.57	2 (3)	36 (59)	0.86
NVP at birth									
No				4 (5)	2 (4)				
Yes			0.57	61 (84)	35 (73)	0.11			0.75
Missing			0.58	8 (11)	11 (23)	0.88			0.31
Maternal characteristics									
Age (mean)	28	29	0.32	28	29	0.32	28	29	0.32
Maternal ARV prior to birth									
Yes							55 (81)	38 (62)	
No			0.80			0.21	10 (15)	13 (21)	0.009
Missing			0.32			0.54	3 (4)	10 (16)	0.18
Marital status									
Single									
Married			0.46			0.35			0.78
Education level									
≥ secondary	16 (19)	10 (31)							
< secondary	69 (81)	22 (69)	0.16			0.79			0.67
Access to water in home									
Yes	19 (59)	61 (74)							
No	13 (41)	21 (26)	0.12			0.41			0.80
Electricity in home									
Yes									
No			0.44			0.87			0.99
Roof									
Iron/tiles	31 (97)	70 (83)							
Grass	1 (3)	14 (17)	0.05			0.25			0.35

**CC: Complete case population; EBF: Exclusive breastfeeding; ABF: Any breastfeeding

APPENDIX B. RESULTS: AIM 3

Base case results

Table 20. Base case results: Point-of-care results, total costs, and pediatric outcomes for early infant diagnosis algorithms

Minimal testing strategies						
		6 month			**9 month**	
	DT	**UN**	**Alere**	**DT**	**UN**	**Alere**
Test results:						
TP	426	372	411	223	213	377
FN	0	0	5	257	142	4
FP	5,286	1,047	16	4,322	213	16
TN	1,031	5,152	6,277	1,818	5,798	6,619
Costs:						
Testing	$220,839.44	$93,030.41	$249,780.17	$179,615.78	$56,904.26	$259,293.25
Counseling	$18,052.14	$11,364.86	$10,075.04	$16,109.78	$10,164.89	$10,448.69
Healthcare	$231,283.92	$201,760.81	$223,104.99	$121,062.78	$115,514.89	$204,539.60
Total costs	$470,175.50	$306,156.08	$482,960.20	$316,788.34	$182,584.04	$474,281.54
Costs/infant	$47.02	$30.62	$48.30	$31.68	$18.20	$47.43
Rank	8	3	10	6	1	9
Outcomes:						
Missed diag.	1,293	1,402	1,353	1,321	1,507	1,190
YLL	25,153	26,328	26,040	27,554	28,596	23,046
YLD	272,551	284,553	281,880	299,822	309,210	250,233
DALY	297,705	310,881	307,921	327,377	337,806	273,279
Rank	15	19	18	20	21	12
Aggressive strategies						
		918 month			**612 month**	
	DT	**UN**	**Alere**	**DT**	**UN**	**Alere**
Test results:						
TP	1,512	1,586	1,227	1,400	1,200	1,359
FN	244	130	4	0	189	19
FP	5,010	195	18	7,124	2,053	32
TN	10,275	15,255	14,645	9,699	15,035	16,195
Costs:						
Testing	$281,075.05	$175,660.60	$599,814.32	$354,825.39	$235,797.89	$664,861.85
Counseling	$33,790.31	$26,884.09	$24,239.70	$38,437.99	$31,071.88	$26,869.50
Healthcare	$820,714.74	$861,230.30	$666,079.50	$760,045.59	$651,476.89	$737,784.61
Total costs	$1,135,580.10	$1,063,774.99	$1,290,133.52	$1,153,308.98	$918,346.67	$1,429,515.96
Costs/infant	$113.56	$106.38	$129.01	$115.33	$91.81	$142.95
Rank	18	15	19	16	13	20
Outcomes:						
Missed diag.	584	503	473	462	483	459
YLL	22,586	20,796	16,254	19,504	20,554	19,256
YLD	250,935	231,606	180,770	218,103	230,213	215,025
DALY	273,521	252,403	197,025	237,607	250,767	234,281
Rank	13	11	2	8	10	7

Table 20 (continued). Base case results: Point-of-care results, total costs, and pediatric outcomes for early infant diagnosis algorithms

Minimal testing strategies						
		12 month			**15 month**	
	DT	**UN**	**Alere**	**DT**	**UN**	**Alere**
Test results:						
TP	465	308	438	586	564	688
FN	0	243	4	147	141	1
FP	899	0	14	342	0	8
TN	5,612	6,353	6,226	6,417	6,872	6,938
Costs:						
Testing	$69,379.84	$56,346.84	$249,681.72	$55,716.61	$69,730.75	$291,582.00
Counseling	$11,860.47	$10,158.83	$10,076.25	$11,939.74	$11,509.08	$11,801.72
Healthcare	$252,520.93	$167,187.68	$237,820.59	$318,324.43	$305,966.19	$373,726.37
Total costs	$333,761.24	$233,693.35	$497,578.57	$385,980.78	$387,206.01	$677,110.09
Costs/infant	$33.38	$23.37	$49.76	$38.60	$38.72	$67.71
Rank	4	2	11	7	5	12
Outcomes:						
Missed diag.	1,163	1,151	1,313	733	704	689
YLL	23,481	25,500	25,659	20,201	19,423	18,255
YLD	255,245	278,594	277,974	223,359	215,003	201,700
DALY	278,726	304,094	303,634	243,559	233,526	219,955
Rank	14	17	16	9	6	5
Aggressive strategies						
		615 month				
	DT	**UN**	**Alere**			
Test results:						
TP	1,504	1,262	1,393			
FN	146	142	12			
FP	6,544	2,053	33			
TN	9,902	14,640	16,427			
Costs:						
Testing	$337,758.91	$235,440.08	$675,125.29			
Counseling	$37,554.88	$30,637.45	$27,286.82			
Healthcare	$816,478.69	$684,898.36	$756,076.68			
Total costs	$1,191,792.48	$950,975.89	$1,458,488.80			
Costs/infant	$119.18	$95.08	$145.85			
Rank	17	14	21			
Outcomes:						
Missed diag.	160	163	228			
YLL	17,650	16,014	15,636			
YLD	200,342	182,118	176,952			
DALY	217,992	198,132	192,588			
Rank	4	3	1			

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