PEDIATRIC HIV DIAGNOSIS & LABORATORY MONITORING

REPORT OF A FORUM FOR COLLABORATIVE HIV RESEARCH WORKING GROUP MEETING

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FORUM FOR COLLABORATIVE HIV RESEARCH

DEPARTMENT OF PREVENTION AND COMMUNITY HEALTH
THE GEORGE WASHINGTON UNIVERSITY
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Special thanks go to the project coordinator, Ipsita Das, without whose expert coordination the project would not have become a reality.

EXECUTIVE SUMMARY

Early diagnosis of HIV status in infants is critical to guide medical management. In the resource-limited setting, an estimated 2.3 million infants and children are HIV infected. The course of HIV disease progression is rapid in perinatally infected children, with approximately half the children dying by 2 years of age if no treatment or prophylactic interventions are in place. The current standard for diagnosis of HIV in adults, HIV antibody tests, cannot be reliably used in infants until they are approximately 18 months of age because of the passive transfer of maternal antibodies. Other HIV diagnostic technologies, such as those based on nucleic acids, are available but their cost, complexity and infrastructure requirements severely restrict their use in most settings.

The recent expansion of antiretroviral therapy programs, as well as programs to prevent mother to child transmission, highlights the urgency for rapid, early and reliable HIV diagnosis in infants. Moving the field forward will require collaboration and coordination among bilateral and multilateral programs, national and regional health ministries, clinicians, public health experts, advocacy community and industry.

The Forum for Collaborative HIV Research convened an international expert group representing all relevant stakeholders in order to:

- Provide a scientific forum to facilitate:
    - Exchange of information on infant and childhood HIV diagnosis and pediatric HIV treatment monitoring
- Identify gaps in knowledge and needed research to address these gaps
- Catalyze and jumpstart the development of infant HIV diagnosis and antiretroviral treatment monitoring tools
- Support and foster collaborative operational projects on infant diagnosis and monitoring for use in resource-limited settings

Children with HIV have been neglected by and large in the rapid scale up of ART programs. In 2005, there were approximately 700,000 new cases of HIV infection in children and 570,000 children died from HIV/AIDS.

HIV is particularly aggressive in children, with mortality rates of 30% at year 1, 50% at year 2 and 60% at year 5. Early infant diagnosis could play a major role in averting excess morbidity and mortality. Currently the majority of children enters care and treatment programs through pediatric consults and is usually quite sick, with over 75% being severely immunocompromised at the time of initiating ART.

The WHO is developing technical recommendations providing global tools and guidance for HIV diagnosis and treatment monitoring in infants and young children. In WHO-sponsored regional consultations, all regions have agreed that active case reporting of pediatric HIV is required.

The WHO's recently released new technical recommendations, including infant diagnosis, are included in the antiretroviral guidelines, and include recommendations for the use of cotrimoxazole, revised clinical and immunological staging, revised age related thresholds for initiation of antiretroviral therapy and clinical and immunological monitoring. These guidelines are based on a public health approach to diagnosis. The WHO has also issued a simplified algorithm for establishing the presence of HIV infection in infants and children in resource-limited settings to enable care and initiation of antiretroviral therapy.

The WHO is developing a guidance note for early infant diagnosis for policy makers, programmers and national programs regarding considerations for the most appropriate testing platform and the appropriate test.

Tom Denny, in collaboration with Jeanne Brosnan, developed a model for market projections to support program development and policy. Using the model's

assumptions, the cumulative ten year market value for CD4 testing is almost 300 million dollars.

Nucleic acid testing (viral RNA or DNA PCR) is considered the gold standard for infant diagnosis and the most widely used assays for infant diagnosis, even in the resource-limited setting but these technologies are usually restricted to the reference laboratories. Nucleic acid testing of dried blood spots has contributed significantly to increased access to testing.

The ultrasensitive p24 antigen assay uses less complex technology and has lower costs compared to PCR. Results from different studies indicate that the assay performs very well for infant diagnosis in children older that 7 days of age.

The Cavidi RT viral load assay has also been evaluated for pediatric diagnosis and there was good correlation with the NucliSens assay.

Immunological assays have been more useful for monitoring the status of the immune system and disease progression than for infant diagnosis. However, the direct and indirect effects of HIV on the immune system could potentially be useful surrogates for the diagnosis of HIV infection in infants.

Differences in CD4 cell counts between HIV infected and uninfected newborns can become apparent as early as one week after birth. However, a CD4 cut-off has not been established for diagnosis of HIV infection. Another potentially complicating factor is that normal CD4 cell counts may differ based on race, gender, ethnicity and geography.

A study from Zimbabwe suggests that it may be possible to diagnose HIV infection using CD4:CD8 ratio. Infants who were PCR positive had lower CD4 cell count, higher CD8 cell counts and a lower CD4:CD8 ratio compared to PCR negative

infants. There may be other potential immunological markers for HIV infection including phenotypic markers of lymphocyte activation and differentiation and serum immunoglobulins.

The current clinical practice "low resource setting protocol" is to follow all HIV exposed infants for one year; provide cotrimoxazole prophylaxis six weeks after birth; and to perform an HIV ELISA test one year after birth. However, in a well-established well-resourced urban clinic in Johannesburg, up to 85% of HIV exposed infants are lost to follow up by one year of age. Furthermore, 38% if HIV infected infants die by one year of age.

Infant diagnosis has become a priority for PEPFAR in 2006. All twenty PEPFAR countries have at least one laboratory with PCR performing capacity and almost all of the countries have active PMTCT/pediatric or PEPFAR laboratory grantees/implementing partners. The goal is to establish a unified plan of support and coordination for infant diagnosis.

PEPFAR has two approaches to infant diagnosis. For early diagnosis, the approach is to use dried blood spots with DNA PCR and for final diagnosis EIA rapid testing. PEPFAR has established an infant diagnosis working group, which is developing a core package of support. The working group's goals for 2006 include: standardize rapid testing for exposed symptomatic children who are over 18 months of age; finalize the PEPFAR guidance and support package for early infant diagnosis; and support scale-up and expansion.

A pilot program for early infant diagnosis was started in Botswana using PCR on dried blood spots. Two hundred and fifty nurses, midwives and doctors were trained in dried blood spot collection. One thousand nine hundred and seventeen HIV exposed infants were tested between the ages of 6 weeks to 18 months. The overall HIV infection rate was 6.7%. The average turnaround time was 9 days from

sample collection to receiving the result in the clinic. Some of the problems encountered in this pilot project included sample collection issues, mostly around labeling errors. One other issue noted was that blood obtained from a femoral stick was most likely to produce a poor sample due to clotting or hemolysis.

Another pilot program for early infant diagnosis was established in Rwanda. Three thousand five hundred and forty-nine children were seen at follow up visits, of whom 177 were determined to have been exposed to HIV. Twelve percent of the children were found to be HIV infected. The turnaround time between blood draw and receipt of results at the site was 17 days.

Kenya has established a program linking infant diagnosis to treatment. PMTCT program sites and health clinics provide the entry point for infant diagnosis. PMTCT programs can help identify HIV exposed children and establish HIV infection status as well as provide systematic follow up.

The best time to test for infant diagnosis, based on a review of data from different studies as well as logistical issues such as child vaccination schedules, appears to be a single DNA PCR test at 6-8 weeks or two tests, one of which is done at day 30 or later are sufficient to predict non-infection in non-breastfed infants. The ideal time point for diagnosing infection will vary depending on whether access to antiretroviral therapy exists, and on whether the infant is being breastfed.

The meeting participants issued some recommendations on research and operational questions that might help expand and lead to more rapid implementation of early infant diagnosis programs. These include:

- Immunological approaches and research questions
    - Validate the role of CD4/CD8 ratio for infant diagnosis based on the data that is already available

  - Evaluate the role of CD4 counts in screening for HIV exposed infants

- Antibody-based testing approaches and research questions
  - At what age can an ELISA test be reliably used for diagnosis of older children?
  - Evaluate the role of rapid tests for infant diagnosis (including at what time point and what age group)
  - Evaluate the role of rapid tests for HIV exposure screening in infants, including those who were breastfed (i.e. at what time point can HIV infection be ruled out)

- Virologic approaches and research questions
  - Issue recommendations on the role of ultrasensitive p24 antigen test for HIV diagnosis (are more evaluations required?)
  - Streamline nucleic acid extraction from DBS (higher throughput and lower cost)
  - What is the best time for virologic testing in a breastfed infant and in a non-breastfed infant?
  - How soon after weaning can a negative HIV PCR or antigen result be confidently reported as negative?
  - Is there a way to determine if an infant has truly weaned and is no longer exposed to breast milk?

- Programmatic and operational approaches and research questions
  - Establish standard operating procedures for infant diagnosis
  - Establish linkage between testing and immunization visits
  - Establish linkages between the different health centers for the mother and infant (PMTCT programs, child health services, ART programs etc.)

  - Operationalize the use of dried blood spots
  - Develop algorithms for early infant testing, including when infants should be tested
  - Examine how to reduce the loss to follow up rate
  - Enhance counseling materials, especially for stigma and disclosure issues
  - Develop a mechanism that allows the healthcare worker to know if the child has been exposed to HIV
  - Develop projections and estimations of short and long term needs for procurement

- Training, capacity building, laboratory set-up
  - Develop training manuals for the different testing technologies
  - Establish external quality assurance programs
  - Establish guidelines on what National Reference Laboratories need to have in place to demonstrate that they can perform quality PCR for infant diagnosis
  - Expand laboratory capacity and the training of healthcare workers to meet the demands of the rollout of infant diagnosis programs
  - Evaluate mechanisms to reduce the turnaround time between drawing the sample and receiving the test result
  - Introduce independent validation of performance for the different test platforms (this should include testing in the conditions where it will be used)

Workshop participants discussed the next steps for the Forum working group on infant diagnosis.

The following priority areas were identified:

- CD4/CD8 ratio (a working group was formed at the meeting to try and validate the role of CD4/CD8 ratio for early infant diagnosis using data from the US as well as international cohorts)

- Compilation of data available on normative values across populations

- Working group to develop consensus on virologic questions:
  - Clarify the role of rapid testing and develop recommendations for its implementation +/- breastfeeding
  - Discuss issues associated with pre-exposure to PMTCT interventions and their possible impact on subsequent treatment of children
  - Discuss integration of ongoing serologic testing programs with virologic approaches
  - Identify ways to capture the experience from different programs and sponsors to inform guidelines and policy; inform what is programmatically efficient
  - Develop consensus on p24 antigen assay: is there a need to keep this going, and if so, who will champion it?
  - Maintain the networking role of the Forum infant diagnostic group to provide partnership and discussion platforms beyond individual programs; provide a bridging mechanism between research and programmatic activities
  - Critically review the currently available PCR data
  - Develop a summary table indicating current technologies, summarizing level of experience, appropriateness for different settings

THE NEED FOR EARLY HIV DIAGNOSIS IN INFANTS

Early diagnosis of HIV status in infants is critical to guide medical management with the goal to reduce excess morbidity and mortality in the pediatric population. In resource limited settings, an estimated 2.3 million infants and children are HIV infected. The course of HIV disease progression is rapid in perinatally infected children with approximately 50% dying by 2 years of age if no treatment or prophylactic interventions are put into place. Laboratory based HIV diagnosis is important because of the lack of specificity of clinical signs and symptoms. The current standard for diagnosis of HIV in adults and older children are antibody detection tests that are relatively inexpensive and technologically simple to implement. However, because of the passive transfer of maternal HIV-1 antibodies across the placenta, tests that detect HIV antibodies are not reliable in infants until approximately 18 months of age. Other HIV diagnostic technologies, such as those based on viral culture, detection of viral nucleic acids or proteins, are available but their cost, complexity and infrastructure requirements severely restrict their use in most settings.

The rapid expansion of programs to reduce mother to child transmission of HIV plus the recent recognition by bilateral and multilateral programs that antiretroviral treatment for HIV infected infants and children needs to become a priority and part of a family centered approach to care and treatment, has increased the urgency to implement services for the early and reliable HIV diagnosis in infants as part of a package of services that also includes: support for infant feeding, cotrimoxazole prophylaxis, regular clinical evaluations in the context of Maternal Child Health programs in resource-limited settings. Moving the field forward will require collaboration and coordination among bilateral and multilateral programs, national and regional health ministries, healthcare providers, clinicians, community-based organizations, public health experts, advocacy community and industry.

The Forum for Collaborative HIV Research, with special support from the Global AIDS Program (GAP) of the Centers for Disease Control and Prevention (CDC), established a Pediatric Diagnosis and Monitoring Working Group with the goal to convene an international expert group representing all relevant stakeholders in order to:

- Provide a scientific forum to facilitate:
  - Exchange of information on infant and childhood HIV diagnosis and pediatric HIV treatment monitoring
- Identify gaps in knowledge and needed research to address these gaps
- Catalyze and jumpstart the development of infant HIV diagnosis and antiretroviral treatment monitoring tools
- Support and foster collaborative operational projects on infant diagnosis and monitoring for use in resource-limited settings

UNAIDS estimates that approximately 40 million people were living with HIV in 2005, the vast majority in sub-Saharan Africa and Asia. This includes 2.3 million children under the age of 15, of whom about 2 million live in sub-Saharan Africa. Over 90% of these children acquired HIV from their mothers either during pregnancy, delivery or postnatally through breastfeeding. PMTCT programs have nearly eliminated perinatal HIV infection in the developed world. In contrast, only 8% of pregnant women are currently receiving ART for PMTCT in the resource-limited setting. Furthermore, 35-40% of HIV infected mothers who breastfeed their infants transmit HIV to their babies by this route. In 2005, there were approximately 700,000 new cases of HIV infection in children and 570,000 children died from HIV/AIDS.

Children with HIV have been neglected by and large in the rapid scale up of ART programs: treatment targets have not been set for children and child focused national responses have been minimal. Infrastructure for the management of children is still lacking as are healthcare providers with expertise in pediatric care.

HIV is particularly aggressive in children, with mortality rates of 30% at year 1, 50% at year 2 and 60% at year 5. HIV diagnosis is problematic in children under the age of 18 months due to the non-specific clinical disease presentation, unreliability of HIV antibody tests and the expense and complex infrastructure required to perform PCR tests. Children do respond well to ART {Fassinou, 2004 #17}{Matida, 2004 #16}. However, optimal treatment monitoring of children under six years of age requires CD4 percentage rather than absolute CD4 cell counts and not all commonly used CD4 assays are adaptable for this.

Table 1 summarizes the current coverage, estimated need for 2010 and estimated costs based on prevention, care and support. As can be seen, currently, all

prevention efforts are affected by extreme coverage deficits, with coverage for children ranging from 1 to 3%.

The pediatric burden of disease is very difficult to estimate. Data to inform an estimated calculation for targeting care and treatment needs are scant, if not completely lacking. Furthermore, there is a paucity of scientific data on effective models for delivery of pediatric care in resource-limited settings. Nevertheless, estimates of the need for antiretroviral treatment and cotrimoxazole prophylaxis have been calculated based on data provided by UNAIDS and UNICEF to the Institute of Child Health. These are shown on Table 2.

Currently, the majority of children enter care and treatment programs through pediatric consults, such as TB clinics, nutritional rehabilitation units, pediatric wards, schools and orphanages. Many of the children entering care and treatment programs are quite sick; nearly half enter at an advanced clinical stage of disease and over 75% are severely immunocompromised at the time of ART initiation.

Early infant diagnosis could play a major role in averting excess morbidity and mortality. In many settings, the window of opportunity for testing is brief, before the child is lost to follow up.

But HIV diagnosis in newborns and infants is very challenging, for reasons already described: the unreliability of antibody-based tests and the expense and complexity of nucleic acid based tests. The use of dried blood spots allowing transport of samples to regional or central laboratories will increase the availability of nucleic acid based tests, as demonstrated in scaled up programs in countries such as South Africa, Rwanda and Botswana.

A number of issues need to be resolved before scale up becomes a reality in many countries, including: training, establishing standard operating procedures for infant

diagnosis, coordinating the role of the national government vis-a-vis the donor community and engaging the manufacturers in efforts to reduce cost while increasing availability.

POLICY AND PROGRAM NEEDS

The WHO is developing technical recommendations providing global tools and guidance for HIV diagnosis and treatment monitoring in infants and young children [www.who.int/hiv/mediacentre/fs_2006guidelines_paediatric/en/index.html]. The major issues at the policy, program and facility level are:

- Policy
  - Principles for HIV testing in infants and children; informed consent, disclosure, confidentiality
  - Need to provide services to children
  - Cost and equity of access

- Program Level
  - Which test platform, testing strategies, test kits, algorithms, how to review and validate them
  - Definitions of HIV infection
  - Burden of disease
  - Monitoring and evaluation of testing
  - Laboratory elements and quality assurance guidance for testing modalities
  - Models and program approaches for service delivery
  - Regular periodic independent assessment of performance characteristics of the platform/test/algorithm
  - Access to bulk purchase and procurement agreements
  - Secure consistency of supplies

- Facility Level
  - Guidance on what service providers should need to know, counseling and associated package
  - Training and tools to support this

Currently, few sensitive and specific testing algorithms exist. Risk of infection is frequently not recognized, and if it is, referral for diagnostics testing does not always follow. Furthermore, the linkages across the various services for antiretroviral treatment, PMTCT programs, HIV testing and counseling and child health services are poor or non-existent.

The more widespread implementation of pediatric diagnosis and monitoring requires simple programmatic oriented technical recommendations, projections and estimation of short and long term needs to allow planning and procurement, reduced prices for the tests and independent validation of performance for the different test platforms. Considerable progress towards achieving these goals could be made through better and more efficient use of the current technologies, such as antibody testing and viral load technologies, where implemented. Policy and program directors need to ensure that technical uncertainties do not lead to total shutdown of infant diagnosis programs. Additionally, simple, tiered programmatic approaches, special attention to registration and regulatory obstacles, and a clear set of priority deliverables need to be developed and/or implemented.

In WHO-sponsored regional consultations, all regions have agreed that active case reporting of pediatric HIV is required. This will be assisted by common laboratory based case definitions. Additionally, programs need to commit to the monitoring of HIV-free survival.

The WHO's recently released new technical recommendations, including infant diagnosis, are included in the antiretroviral guidelines, and include recommendations for the use of cotrimoxazole, revised clinical and immunological staging, revised age related thresholds for initiation of antiretroviral therapy and clinical and immunological monitoring. These recommendations are based on a public health approach to diagnosis, incorporating acknowledgment of ongoing

exposure to HIV (e.g. through breastfeeding). In the event that exposure to HIV has been discontinued, testing for HIV infection by the best age appropriate test available six weeks after the complete cessation of breastfeeding is recommended. Within the public health context, one positive virologic test in a child six weeks of age or older with documented HIV exposure is sufficient for purposes of clinical management and antiretroviral treatment initiation, thus alleviating the need for confirmatory testing.

Table 3 outlines a summary of recommendations on methods for establishing the presence of HIV infections in infants and children.

The WHO has recently issued a simplified algorithm for establishing the presence of HIV infection in infants and children in resource-limited settings to enable care and start antiretroviral treatment, as shown in Figure 1.

The WHO has also issued clinical guidelines for presumptive diagnosis of severe HIV in an HIV exposed infant in the absence of virological testing:

Seropositive infant:

- Symptomatic with two or more of the following:
  - Oral thrush;
  - Severe pneumonia*;
  - Severe wasting/malnutrition*;
  - Severe sepsis*

- Other factors to support diagnosis of severe HIV include:
  - Recent HIV-related maternal death; or

* As defined by IMCI

  - Advanced HIV disease in the mother; or
  - Documented history of no maternal or infant ART for MTCT

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

Table 4 illustrates the WHO guidelines for laboratory parameters for monitoring children and infants prior to initiating antiretroviral therapy and during treatment.

Some of the key issues that still need to be resolved for infant diagnosis include:

- Virological Tests
  - Which assay is most appropriate for infant diagnosis: DNA PCR, RNA PCR or p24?
  - Does confirmation of HIV infection require a different assay?
  - Will the assay detect all HIV subtypes?
  - What is the optimal time for infant testing?
  - What are the laboratory capacity requirements?
  - Have the quality assurance requirements been met?
  - Does the assay allow the use of dried blood spots so that samples can be sent to a central laboratory?

- Antibody Tests
  - How often should testing be done and how soon after birth?
  - Is there a window period post breastfeeding?
  - Can antibody tests be used as a screening tool?

- Presumptive Clinical Diagnosis
  - Validation is urgently required

- Simple algorithms are needed for less experienced healthcare workers to recognize HIV exposed and infected infants
- Specificity improvement with the addition of simple add on tests such as hemoglobin or CD4 needs to be established

Participants recognized that the entire area of counseling, informed consent, and disclosure may be an obstacle to large rollout programs for early infant diagnosis. Progress in this area needs to occur in parallel with technical progress and program implementation.

The WHO is developing a guidance note for early infant diagnosis for policy makers, programmers and national programs regarding considerations for the most appropriate testing platform and the appropriate test. This guidance note will be available soon.

As stated above, global needs assessment for pediatric HIV care and diagnostics is difficult. Nevertheless, projections are needed for program planning and for engaging industry commitment. Tom Denny, in collaboration with Jeanne Brosnan, modeled market projections for the purposes of illustrating the potential global market share, to support program development and policy. The WHO's 3 by 5 program forecasts that, globally, over ten million people will be on ART by the year 2010. Most of the ten million people, however, will live in areas without stable electricity, with poor water supply and generally poor infrastructure. Different technologies will be needed based on these different markets, environments and different levels of care.

The model used seven million children who currently need PCP prophylaxis and antiretroviral therapy as projected by the WHO and the pediatric laboratory evaluation/monitoring schedule from the 2005 Columbia Clinical Manual, Care and Treatment of HIV/AIDS in Resource-Limited Settings. The CD4 monitoring frequency includes measurements at the time of diagnosis, then every two months during the first six months of life, then every three months from 6-18 months of age and then every six months thereafter. This model also assumes a 5% net gain, per year, in the number of CD4 count tests required (10% growth rate and 5% death rate), that access to CD4 testing will increase from the current 4% to 50% in five years and plateau at 60%, that the average number of test required is two per year and that the cost per test is $4. Based on these variables, at year ten, the total market value for these tests is approximately $55 million and this may actually be an underestimation due to the assumptions that each child will be tested for CD4 counts only twice a year. The cumulative ten year market value for CD4 testing is almost 300 million dollars.

The Global Health Technology group, supported by the Gates Foundation in collaboration with the Rand Corporation is developing a model to determine the

impact of diagnostics on malaria, acute lower respiratory infections, tuberculosis, sexually transmitted infections and HIV. UNICEF is hoping to establish a guaranteed fund that can be used to purchase pediatric therapies and diagnostics.

The Clinton Foundation has also been working with the Essential Health Technologies group at the WHO to establish a pre-qualification like system for diagnostics. Establishing standards that can be met by the existing diagnostic manufacturers, and standards for new technologies and products is essential for program quality. However, compared to drugs, pre-qualification for diagnostics is less of an incentive due to the lack of regulation of diagnostics in most countries, thus allowing companies to market their diagnostic test without regulatory approval.

Push & Pull

Market projections are essential for the "pull" mechanisms that drive research and development for new technologies. Together with assurances from programs such as the Global Fund to Fight AIDS, Tuberculosis and Malaria, the PEPFAR program, the Clinton Foundation, and the WHO's 3x5 program, these encourage commitments from industry to develop and supply the needed products.
"Push" mechanisms help drive development of new technologies through advocacy, negotiation and supportive programs, such as the NIH's SBIR program. The Gates Foundation and the Clinton Foundation have been working with the Essential Health Technologies group at the WHO to help reduce the price of diagnostics. However, one concern with reducing pricing too soon is that this may reduce some of the incentive for diagnostic companies to develop a new technology that may be more appropriate for resource-limited settings.

STATE OF THE ART TECHNOLOGIES AND GAPS

Susan Fiscus provided an overview of Infant HIV diagnosis based on detection and quantification. The limitations of some of the existing technologies restrict their use to the reference centers, as is true for the nucleic acid based tests, while others may be better suited for the provincial or district level health centers. The availability of dried blood spots has contributed significantly to increased access to testing.

Dried blood spots (DBS) have been used to collect blood for public health purposes for over 40 years. DBS have the potential to reduce the cost of viral load testing by simplifying the collection, storage and shipping of the sample. Not all filter paper used for collection of DBS is the same and may contribute to laboratory misdiagnosis.

Nucleic acid testing (viral RNA or DNA) is considered the gold standard for infant diagnosis and the most widely used assays for infant diagnosis, even in the resource-limited setting. The commercially available RNA and DNA PCR assays are highly sensitive and specific for early detection of HIV infection.

Two Real Time PCR assays have been developed: the Real Time DNA PCR and the Real Time RNA assays. Both have the disadvantage of high purchasing costs: the estimated equipment cost will be in the range of $30,000 – $40,000. Both are probably best suited for centralized testing in a reference laboratory with highly skilled technician and adequate infrastructure. They use home brew primers and probes, will likely be able to detect all clades of HIV and should be compatible for use with dried blood spots. Both assays are closed systems. The DNA test appears to be sensitive down to 10 copies of DNA per test, uses AmpErase uracil-N-glycosylase (UNG) to minimize contamination and includes an internal control, whereas the RNA test has a lower limit of quantification (300 copies of HIV RNA per mL of blood) and uses an external standard curve rather than an internal standard. The advantages are that it is high throughput and very reproducible, with a reported cost of approximately $12 per test. The Real Time RNA PCR has been successfully implemented Abidjan, Cote d'Ivoire {Rouet, 2005 #1}.

The HIV DNA PCR assay is now considered the gold standard for early pediatric HIV diagnosis. This assay allows for a small amount of DNA to be amplified exponentially. Gayle Sherman and colleagues have investigated the utility of the Roche HIV DNA PCR version 1.5 {Sherman, 2005 #14} for infant HIV diagnosis using dried blood spots. The study included a simplified and less costly extraction procedure. The dried blood spots for this study were obtained from infants 6 weeks of age using Whatman #1 paper, which had been stored for 9 – 19 months at room temperature without a desiccant. Compared to the DNA PCR, the sensitivity of the adapted assay demonstrated 100% sensitivity and 99.6% specificity.

The ultrasensitive p24 assay uses less complex technology and has lower costs compared to PCR. Heat dissociation allows detection of HIV-1 p24 antigen with very reproducible results. Although the buffer included as part of the kit version appears to be less sensitive than a buffer developed by Jorg Schupbach of the Swiss National Center for Retroviruses that is commonly used with this assay when performing viral load testing. Due to typically high viral loads observed in infected infants, the external buffer does not appear to be needed for perinatal diagnosis of HIV.

The results from the North Carolina and New York City Perinatal AIDS Collaborative Transmission Study (PACTS) study using the ultrasensitive p24 antigen assay indicate that the assay performs very well for infant diagnosis in children older than 7 days as shown in Table 6.

A study of an adapted ultrasensitive p24 antigen assay using dried blood spots on Whatman #1 paper, demonstrated good reproducibility with a sensitivity of 98.8% and specificity of 100% compared to the NASBA RNA and Roche RNA and DNA assays {Patton, 2006 #2}. There was also correlation between plasma viral load and the dried blood spot ultrasensitive p24 antigen assay. All of these specimens were

tested within six weeks of the blood draw – there was reduced sensitivity when the specimens were tested twelve weeks after the blood draw, suggesting that the proteins are not stable on the dried blood spot over a longer period of time.

Table 6 lists the different studies using heat dissociated p24 antigen for infant diagnosis.

The Cavidi RT viral load version 1.0 assay was studied in children in Addis Ababa {Seyoum, 2006 #3} for viral load monitoring. Only 0.2mL of plasma was used instead of the recommended 1mL, however, the correlation with the NucliSens assay was good.

Future Options

Helen Lee's group at the University of Cambridge recently published on their multiplex dipstick assay, which can detect HBV, HCV and HIV nucleic acids {Dineva, 2005 #19}. Extraction and amplification are required and the detection is with the dipstick, which takes approximately 15 minutes. The detection limits reported are 50 IU HBV DNA, 125 IU HCV RNA and 500 IU HIV RNA. The availability of this point of care assay will mean that infant diagnosis will be able to be performed in very remote sites and there will be less need to train healthcare workers on the details of using dried blood spots and shipping the samples to a reference or central laboratory.
Medecins sans Frontiers recently sponsored a roundtable (January 2006) to define the specifications for the multiplex dipstick test being developed by Helen Lee, as described above. The dipstick, once developed, could potentially be used as a diagnostic test as well as for treatment monitoring. The reagents for the dipstick will not require a cold chain and can be performed by people with limited training. The participants of this roundtable recommended a diagnostic threshold for infant diagnosis, and that this threshold be set at 10,000 copies/mL HIV RNA.

THE ROLE OF IMMUNOLOGY AND IMMUNOLOGIC MARKERS IN PEDIATRIC HIV AND INFANT DIAGNOSIS

Savita Pahwa provided an overview of the role of immunological markers in HIV diagnosis. As stated previously, in infants, the presence of HIV antibodies is indicative of exposure to HIV and not of HIV infection because of the passive transfer of maternal antibodies (IgG) through the placenta, which can persist for up to 18 months of age.

Immunological assays have been more useful for monitoring the status of the immune system and disease progression than for infant diagnosis. However, the direct and indirect effects of HIV on the immune system could potentially be useful surrogates for the diagnosis of HIV infection in infants.

As with all diagnostic assays, an immunological marker or function in HIV-exposed infants should be able to distinguish them from HIV exposed uninfected infants and from non-HIV exposed uninfected infants. Therefore, any potential immunological assay would require establishment of 'normal' age-appropriate values for the uninfected population.

Immunological assays could potentially be developed based on pathogenesis of HIV disease for infant diagnosis. These would include assays that identify immune cell depletion, identify HIV-associated cellular activation/immune dysregulation or detect HIV-specific immune responses.

Differences in CD4 cell counts between HIV infected and uninfected newborns can become apparent as early as one week after birth {Shearer, 2000 #12}. However, a CD4 cut-off has not been established for diagnosis of HIV infection and the question whether CD4 percentage or absolute CD4 cell count is the most appropriate measure is still debated. Another potentially complicating factor is that normal CD4 cell counts may differ based on race, gender, ethnicity and geography.

A recently published study from Zimbabwe suggests that it may be possible to diagnose HIV infection using CD4:CD8 ratio. One hundred and thirty-seven breastfeeding infants from two independent prospective studies – short course AZT and the Pediatric AIDS Clinical Definition (PACD) - under the age of two were enrolled in the study. A total volume of 2mL whole blood was collected from the infants to obtain T cell subset profiles and for DNA PCR analysis. Infants who were PCR positive had lower CD4 cell count, higher CD8 cell counts and a lower CD4:CD8 ratio compared to PCR negative infants. The results from this study are shown in Tables 8 and 9 {Zijenah, 2005 #13}.

Perinatal HIV transmission and HIV infection in infants have also been shown to be associated with other phenotypic markers of lymphocyte activation and differentiation. In the PACTG 185 study of 215 women and 192 infants, women who did not transmit HIV to their infants had higher CD4 counts and lower CD8 and CD38 cell counts {Lambert, 2005 #21}. Infants who were not HIV infected had higher CD3, CD4 and naïve CD4 cell counts. Infants infected with HIV had higher total CD8, CD8 HLA DR, CD45RA HLA DR and CD28 HLA DR cell counts.

Serum immunoglobulins may also be a potential marker for HIV infection. In a prospective five year study, IgG, IgA and IgM levels were significantly higher in HIV infected children than uninfected children {Shearer, 2000 #12}.

The sensitivity of the HIV IgA ELISA and p24 antigen ELISA for HIV diagnosis has also been studied {Desai, 2005 #22}. Among 42 HIV DNA PCR positive children under 18 months of age, 30 were positive by HIV IgA and only 16 were positive by p24 antigen. No false positives among infants who were HIV DNA PCR negative were observed in either assay.

Some potential immunological diagnostic assays based on alterations in peripheral blood cellular composition include reduction in CD4 cells, naïve CD4 cells and CD4/CD8 ratio or increases in CD8 cells and activated CD8 cells. Some potential serum based markers include increases in immunoglobulins such as IgM/IgA and HIV antibodies such as IgA.

Pediatric HIV diagnosis is a mechanism to monitor prevention of mother to child programs. In the clinical context, however, its use in determining the appropriate care for the infant, whether they are HIV exposed and their HIV status is unknown, HIV exposed and uninfected or if they are HIV infected, is most important. Infant diagnosis is also an opportunity to build systems so that other family members can enter care or be tested.

The current clinical practice "low resource setting" protocol is to follow all HIV exposed infants for one year; provide cotrimoxazole prophylaxis six weeks after birth; and to perform an HIV ELISA test one year after birth. If the ELISA is positive, further tests are performed. The urgency for early infant diagnosis based on the high rates of loss to follow-up and rapid death rates is illustrated by Dr. Sherman's experience in Johannesburg. In a well-established, well-resourced urban clinic, up to 85% of HIV exposed infants are lost to follow up by one year of age. Furthermore, 38% of HIV infected infants die by one year of age. This means that it is almost impossible to evaluate the PMTCT program and that most of the children who need it, do not have access to HIV care.

Gayle Sherman and her group found that efforts to diagnose HIV by clinical assessments lacked sensitivity, especially in infants under 3 months of age. The results are listed in Table 10. As part of the study, the children received ELISA tests at 3 month, 7 month and 12 months of age. At 3 month of age, the ELISA was not able to discriminate between HIV infected and uninfected children. At 7 months of age the presence of some outliers made discrimination between HIV infected and uninfected children difficult. However at 12 months of age, it was very easy to discriminate between infected and uninfected children provided the HIV ELISA optical density (OD) readings were available. In 369 HIV-exposed infants with a median age of 12.1 months, using ELISA OD readings increased the

specificity of an HIV ELISA from 59% to 96% without reducing the sensitivity of 100%.

The 2004 National Antiretroviral Treatment Guidelines from South Africa state that rapid HIV tests should not be routinely performed in children, as they are less reliable in children than in adults. Furthermore, some countries have regulations that state rapid tests may not be used in children under 18 years of age. However a study involving more than 800 children over 18 months of age demonstrated that rapid testing works as well in children as in adults {DeBaets, 2005 #23}. WHO guidance on this issue is urgently required, as many children over 12-18 months of age should be tested and, if necessary, access care and treatment.

Gayle Sherman and her colleagues also evaluated oral fluid HIV ELISA tests (OraSure and Vironostika) and a rapid oral fluid HIV test (OraQuick) compared to a serum HIV ELISA test among children with a median age of 12.2 months (11-18months). The results are listed in Table 11. {Sherman, 2005 #14}

It may be possible to test for HIV infection using oral fluid or blood using an ELISA or a rapid test when a child is 12 – 18 months of age to identify children who are HIV uninfected. Children who are found to be HIV infected will need to receive a confirmatory virological test. It may be possible to start using a rapid test when the child is 9 months of age.

The infant diagnosis program in South Africa is on hold because of concerns that laboratory capacity will be exceeded even though only 16% of children who should be tested are getting tested. Mechanisms for laboratory scale up are urgently needed. Laboratory scale-up, in contrast to clinical programs, should be easier to scale up, as the requirements -- laboratory space, equipment and consumables and technologists -- are more easily met.

Infant diagnosis became a new priority for PEPFAR in 2006. Previously, the focus centered on coverage of prevention of mother to child transmission programs and scale up of care and treatment. The activities of the groups focusing on PMTCT and pediatrics have been joined with the laboratory group. This unites three key areas – monitoring and evaluation, clinical care and capacity building, and will allow for the evaluation of the effectiveness and impact of the PMTCT program, provide an avenue for pediatric clinical care and early treatment and allow for capacity building, national systems and planning.

All twenty PEPFAR countries have at least one laboratory with PCR performing capacity and most have proven research experience in partnership with a US university or other technical partners. Some countries, such as Kenya and South Africa, already have well developed local capacity. Approximately half of the countries have CDC laboratory assignees in-country and eventually all will have active CDC laboratory back-up from Atlanta to support technical assistance to countries and to partners. Almost all of the countries have active PMTCT/pediatric or PEPFAR laboratory grantees/implementing partners. The goal is to establish a unified plan of support and coordination for infant diagnosis.

PEPFAR has two approaches to infant diagnosis. For early diagnosis (6 weeks to 6 or 12 months of age) the approach is to use dried blood spots with DNA PCR and for final diagnosis (over 18 months of age) EIA rapid testing. Strengthening the linkages between the PMTCT programs and the child health programs will be essential for this approach to succeed. One mechanism to help accomplish this is to include the HIV status on the child health card and to build supporting systems to link PMTCT programs with child health programs.

PEPFAR has established an infant diagnosis working group. A consultation meeting was held in July 2005 and the support for country implementation of infant diagnosis was strong. The Roche Amplicor DNA assay was selected as the initial standard assay to be used with dried blood spots. The working group is developing a core package of support and this includes developing clinical and laboratory protocols, standard operating procedures, quality assurance procedures, establishing a training package and video and working with Roche on technical assistance, procurement and to establish a favored pricing program for the DNA PCR assay.

Several operational issues and operational research questions remain to be addressed. These include comparing the different technologies for infant diagnosis and identifying the optimal methodology, identifying the most appropriate time to test and the appropriate time for the confirmatory test, improving follow up and integration with the Expanded Program on Immunization (EPI) and evaluating the role of rapid tests for screening of infants who are 9-12 months of age or older. Another key issue is the challenge of counseling, especially with an early HIV negative test for breastfed infants and the need to develop weaning approaches for the mother. Additionally, increased efforts are needed to train healthcare providers for counseling of parents around stigma and disclosure issues; unaddressed, these could potentially limit the scale-up of early infant diagnosis programs. Linkages also need to be established between programs focusing on behavioral and programs focusing on biomedical interventions.

The PEPFAR working group's goals for 2006 include: standardize rapid testing for exposed and symptomatic children who are over the age of 18 months; finalize the PEPFAR guidance and support package for early infant diagnosis; and support scale-up and expansion.

Dorothy Mbori-Ngacha noted that in Kenya, where the majority of women breastfeed their infant, the approach has been to test for HIV infection using DNA PCR at a time point where transmissions is most likely to have occurred, balanced against the risk for early deaths. The current proposal is to test at the time of the third immunization visit, which usually occurs at around 14 weeks. This was based on some of the local research studies showing that 60-70% of transmissions through breastfeeding have occurred at the 14 week time point (see below). This approach is duplicated in Western Cape in South Africa, where HIV DNA PCR testing is also done at the 14 week time point. However, as noted by Mark Cotton, morbidity in some infants under 14 weeks of age was observed.

Botswana

Botswana is one of the countries hardest hit by HIV and approximately 37.1% of pregnant women are HIV positive. A PMTCT program was initiated in 1999, and has functioned at the national level since 2001. The program includes routine HIV testing of all pregnant women and provides antiretroviral therapy to the mother if her CD4 count is below 200 cells. The program also provides AZT for 12 weeks to the mother and 4 weeks to the infant combined with single dose nevirapine to both the mother and infant, in cases of mothers who do not meet the indication for antiretroviral therapy. Additionally, infant formula is provided for 12 months. A child health card with PMTCT interventions on the card was introduced recently. The Botswana antiretroviral program was started in 2001 and the national expansion of this program was completed in 2004. Approximately 60,000 people are on therapy, of which approximately 3,000 are children. Currently, access to pediatric antiretroviral therapy is only available at a few large sites but this program is expanding to include additional sites.

The local guidelines established in 2005 recommend that all HIV positive children under the age of 12 months be on antiretroviral therapy regardless of CD4 cell count and children over the age of 12 months be on antiretroviral therapy based on CD4 cell counts and/or clinical criteria.

Rwanda

Rwanda, with an HIV prevalence of 5.1%, started a national program for routine early infant testing in September 2005 using PCR testing of dried blood spots, which is available at the National Reference Laboratory. Access to pediatric antiretroviral therapies has also been implemented and is available at various sites around the country. The Ministry of Health estimates that approximately 18,000 infants are born to HIV infected mothers a year and only 11.9% of all HIV exposed children are followed up and tested by 15 months of age.

Kenya

Antiretroviral treatment programs are being scaled up in Kenya but presently less than three percent of adults and children who need treatment are actually on treatment. More than 88% of Kenyan women make at least one visit to an antenatal clinic and 60% of the maternal child health facilities have PMTCT services. Approximately 80% of infants attend clinic at six weeks of age to receive their vaccinations.

Tracy Creek provided an overview of Botswana's program, which succeeded in introducing dried blood spot testing of infants in the country's PMTCT program. The percentage of women receiving PMTCT interventions has grown significantly since 2002 as illustrated in Figure 2.

Based on the detailed PMTCT uptake data, it is estimated that approximately 6% of infants born to HIV infected mothers in the city of Francistown in 2005 were HIV infected. The transmission rate within the national PMTCT program is not known.

Selected sites have been offering early infant diagnosis by PCR on whole blood since 2001; however, the program experienced problems with the turnaround time

and with sample storage at the laboratory. Additionally only a few sites were capable of doing phlebotomy on young children. Dried blood spots are expected to solve the problem with sample storage, transportation and blood collection problems and also allow for wider access to early diagnosis. A pilot program of PCR on dried blood spots was initiated with the goal of: determining the feasibility of dried blood spot collection at government clinics during routine infant care; identifying and solving problems with sample collection; establishing a laboratory quality assurance system; documenting rates of HIV infection among infants; and determining if infants diagnosed early leads to early treatment.

The pilot program on dried blood spots ran from June to December 2005, with ten clinics and the regional referral hospital in Francistown as well as the Botswana-Baylor Children's Center of Excellence in Gabarone participating. Two hundred and fifty nurses, midwives and doctors were trained in dried blood spots collection. The training involved one day of classroom training (review of HIV testing principles, antiretroviral therapy guidelines, pre and post test counseling etc.) and several days of hands-on training at each site. A dedicated technician at the national HIV reference laboratory ran all the tests on a Roche Amplicor PCR version 1.5 with manual extraction. The technician could perform up to 48 samples per day. All of the samples were transported by DHL and the results were reported back via fax. The testing algorithm used in Botswana is shown in Figure 3.

One thousand nine hundred and seventeen HIV exposed infants were tested between the ages of 6 weeks to 18 months. The overall HIV infection rate was 6.7%. The rate was higher at the hospital (12% of 561 infants) than in clinics (4.4% of 1356 infants). Among the 1356 outpatient infants tested in clinics, 99% received at least one intervention for PMTCT, 98% were formula fed, 81% of infants between the age of 3-12 months were on cotrimoxazole and 81% of caregivers received the results of the infant's test. As would be expected, infants who received

no PMTCT interventions were most likely to be HIV infected compared to those who did receive some intervention. Details are provided in Table 12.

The dried blood spot approach to infant diagnosis was acceptable to the medical staff and to mothers, with over 90% of the HIV exposed infants under 12 months of age in Francistown tested during the pilot program. Nurses and midwives were equally adept at collecting dried blood spots with 73% of the infants needing to be stuck just once to collect a sample. Heel sticks appeared to work best for infants between the ages of 1-4 months or weighing less than 6kg and toe sticks worked best for infants between the age of 4-10 months or weighing less than 10kg. Finger sticks may be needed for older children or children weighing more than 10kg.

All of the positive HIV tests obtained in the pilot project were confirmed by a second test on the same sample. No false positives were detected in the clinic and all of the CDC quality assurance samples were correctly reported. The average turnaround time was 9 days from sample collection to receiving the result in the clinic. One issue noted was that blood obtained from a femoral stick was most likely to produce a poor sample due to clotting or hemolysis. Additional problems encountered during the pilot project included sample collection issues, mostly around labeling errors. Labeling errors may be reduced by including names and dates of birth, if allowed, to be put on the label, providing a link to the child health card rather than the current policy in Botswana of anonymity. Attaching the cards to the form as well as the use of stickers and barcodes may also reduce labeling errors.

Encouragingly, HIV-positive children identified through early diagnosis in this pilot project did receive early therapy, although the loss to follow up rate remained high (26%).

Future plans call for a training video that will be distributed by Roche. Discussions with Botswana's Ministry of Health to address the issues around labeling, supply storage and distribution have been initiated. National rollout of early infant diagnosis under the Ministry of Health will start later in 2006 and will use dedicated training teams funded by the US government. Ideally, industry will be able to package a kit with all the necessary supplies required to perform the test on 50 babies.

In Botswana, the train-the-trainer model may not be the most effective way of scaling up this program because of all the complexities involved. Instead, the plan is to use the CDC team from Francistown, who will conduct the training in communities within a day's drive, complemented by nurses and physicians working on a contract basis.

Human resource issues represent the major obstacle to scaling up in Botswana. The country receives substantial funding from PEPFAR, the Gates Foundation and Merck; however, hiring of technicians can take over a year due to lack of local technicians necessitating out of country searches. Reliable equipment maintenance is another major obstacle. Lengthy delays in repair scheduling can cause significant back-logs in sample processing.

In Rwanda, the objectives of the routine early infant testing program are to: identify infants born to HIV infected mother at possible entry points (e.g. routine vaccination, nutrition centers etc.), start cotrimoxazole prophylaxis and initiate antiretroviral therapy if the infant is HIV infected. The Rwandan Ministry of Health coordinated this program and the first phase was started at three centers in Kigali in October 2005. Two of the three centers are in an urban location; two are considered to be health centers and the third is a hospital. There is commitment for rapid scale-up and expansion of this program, which is supported by PEPFAR through various partners.

This program involved counseling mothers to bring their ANC card, which is coded with HIV status, to the pediatric follow up visits. The ANC card is stapled to the vaccination card and this allows the medical staff to determine the HIV exposure status of the child. All exposed children between the ages of 6 weeks and 18 months are tested and started on cotrimoxazole. The dried blood spot preparation is through a heel prick and specimens are sent to the National Reference Laboratory on a weekly basis. Test results are collected at the laboratory weekly. The testing algorithm used in Rwanda is shown in Figure 4.

Between October and December 2005, 3549 children were seen at follow up visits. The HIV status of the mother was known in 2343 children; 177 children were determined to have been exposed to HIV. The majority of these children (95.7%) had been breastfed and half were still being breastfed. The median time of breastfeeding, among children who stopped breastfeeding, was 5.6 months. The turn around time between blood draw and receipt of results at the site was 17 days (range was 9-54 days). Twelve percent of the children were found to be HIV infected. The results are shown in Table 13. Sixty-one percent of the mothers were evaluated for eligibility of antiretroviral therapy, which is offered for people with <350 CD4 counts, during pregnancy. Twenty-one percent of these women were eligible for antiretroviral therapy.

A number of challenges have been encountered to date. The confirmation of a positive test result is time consuming and requires an extra visit. Only four confirmation tests out of the twenty positive test results have been performed and two children have died before a confirmation test could be collected. Three of the initial positives were negative upon confirmation testing; whether this is due to mislabeling, contamination or inadequate quality assurance procedures, is not known at this time.

The Rwandan program will be reviewed after 6 months of operation by which time approximately 500 children will have been tested. Some changes have already taken place, including an immediate clinical evaluation for initiating antiretroviral therapy after the first PCR positive test result. Confirmatory tests will still be done, but the immediate evaluation allows more rapid access to treatment, if warranted. Rwanda has moved towards a policy of offering rapid testing for screening of all children over nine months of age. Information on HIV exposure, HIV test results and feeding practice will be included on the vaccination card.

Future plans call for expansion of sites in Kigali as well as rural sites and adding PCR capacity in a decentralized second laboratory. This will result in additional laboratory capacity by about 25,000 PCR tests per year.

Dorothy Mbori-Ngacha presented on a program in Kenya linking infant diagnosis to treatment. PMTCT program sites and health clinics (including outpatient departments and pediatric inpatient wards) provide the entry point for infant diagnosis. PMTCT programs can help identify HIV exposed children and establish HIV infection status as well as provide systematic follow up, such as cotrimoxazole prophylaxis and nutritional support. However, a mechanism for systematic follow up has not been set up at most of the sites in Kenya. The Kenyan experience confirms the failure of using clinical criteria to identify children under 18 months of age who need antiretroviral therapy.

As in the other examples, major issues remain to be addressed. In Kenya, the current testing algorithm is based on antibody tests to identify HIV exposed children with more targeted use of DNA PCR to identify HIV infection in exposed children. Infants who are healthy but of unknown HIV exposure status should receive an antibody test at six weeks of age or at first contact. All HIV exposed infants receive repeat testing at 12 and 18 months of age. All sick children receive an antibody test when they are admitted to the hospital or at the outpatient clinic if

they have any of the following conditions: failure to thrive, pneumonia, developmental delay or recurrent visits/admissions. Targeted testing of children who have repeated admissions/visits is being studied. The acceptance rate of routine antibody testing has been approximately 90%. At the Kenyatta National Hospital, approximately 50% of the children have been exposed to HIV; the majority of these children are under two years of age. These exposed children receive repeat testing by DNA PCR. Some of the operational issues that need to be put in place include: linking antenatal record and infant record (a new card has been developed and will be piloted later in 2006), human resources (especially in the more rural areas), and integration of services – PMTCT/Maternal Child Health and HIV care and treatment. DNA PCR testing is performed in the PMTCT programs at six weeks of age for healthy infants who are not breastfeeding or at 14 weeks of age for healthy infant who are breastfed. This approach allows for early identification of children infected who are more likely to progress rapidly and is linked to a visit made by the majority of Kenyan women. HIV infected infants will be able to receive care and treatment as per the Kenyan guidelines; HIV negative infants will receive repeat antibody testing at 9, 12 and 18 months of age.

DNA PCR is available in research laboratories, but the technology needs to be established within a network of regional laboratories able to run this assay, while ensuring quality assurance. Logistic plans for sample transport and reporting of results need to be developed. Healthcare workers need to be trained on the infant diagnosis algorithm as well as sample collection and communication of results. Additionally, laboratory personnel need to be trained on good laboratory practices as well as PCR methodology.

Approximately 90% of all mothers attending antenatal clinic as part of the PMTCT program at the Kenyatta National Hospital return for the six week visit, at which dried blood spot samples are taken. As for the previous national program examples, the lengthy turnaround time (in this case due to test batching by the laboratory) has

led to disappointment and frustration for providers. The high rate of rejection of samples is another source of frustration. Increasing the network of providers who send samples to overcome the 'critical mass' barrier is one approach to improve turn-around time.

One ongoing problem in infant diagnosis services is the fact that the mother and the child frequently receive care and treatment in different places; the linkages between these care sites, as well at PMTCT sites, are not well established. Zambia has been piloting a smart card system for electronic medical record follow up, for which the pediatric module is still being developed. This may help provide some of the linkages between the different points of care as well as for patient follow up.

Early infant diagnosis using dried blood spots can be implemented in resource-limited settings as illustrated by the programs from Botswana and Rwanda. However, many barriers still exist and must be overcome before broader implementation can take place:

- Provide adequate training of healthcare workers
- Ensure adequate capacity at the laboratory to perform all the tests that are forecasted
- Establish a mechanism for confirmatory testing
- Link antenatal records and infant records
- Integrate services such as PMTCT programs/Maternal Child health programs with HIV care and treatment programs
- Establish quality assurance of laboratories
- Establish a plan to ship samples to the laboratory

The best time to test for infant diagnosis was discussed at length. Based on a review of data from different studies noted above as well as logistical issues such child vaccination schedules, it appears that a single DNA PCR test at 6-8 weeks or two tests, one of which was done at day 30 or later are sufficient to predict non-infection in non-breastfed infants.

The ideal time point for diagnosing infection will vary depending on whether access to antiretroviral therapy exists, and on whether the infant is being breastfed. For example, if antiretroviral therapy is not available, one suggestion was to test at six months using a rapid test and then use PCR as a confirmation. In Kenya, the recommendation is to test breastfed babies at 14 weeks, based on the rationale of identification of rapid progressors as early as possible so that they may be offered care and treatment, and the fact that this time point will also identify many infants infected through breastfeeding. Testing at 6 weeks will primarily detect infants who are infected intra partum or in utero and not those infected through breastfeeding. However, in many settings, testing at six to eight weeks is preferred because most infants will receive vaccinations at that time. A positive antibody test result at that time would at the minimum provide for cotrimoxazole prophylaxis and HIV care. In Rwanda, on the other hand, children are tested using a rapid test at nine months, which coincides with the measles vaccination. However, this time point may be less than three months since the child was weaned from breastfeeding, and it is not clear whether this is far enough outside the window period to confirm the child as HIV uninfected if the test is negative.

The meeting participants issued some recommendations on research and operational questions that might help expand and lead to more rapid implementation of early infant diagnosis programs. These include:

- Immunological approaches and research questions
  - Validate the role of CD4/CD8 ratio for infant diagnosis based on the data that is already available
  - Evaluate the role of CD4 counts in screening for HIV exposed infants

- Antibody-based testing approaches and research questions
  - At what age can an ELISA test be reliably used for diagnosis of older children?
  - Evaluate the role of rapid tests for infant diagnosis (including at what time point and what age group)
  - Evaluate the role of rapid tests for HIV exposure screening in infants, including those who were breastfed (i.e. at what time point can HIV infection be ruled out)

- Virologic approaches and research questions
  - Issue recommendations on the role of ultrasensitive p24 antigen test for HIV diagnosis (are more evaluations required?)
  - Streamline nucleic acid extraction from DBS (higher throughput and lower cost)
  - What is the best time for virologic testing in a breastfed infant and in a non-breastfed infant?
  - How soon after weaning can a negative HIV PCR or antigen result be confidently reported as negative?
  - Is there a way to determine if an infant has truly weaned and is no longer exposed to breast milk?

- Programmatic and operational approaches and research questions
  - Establish standard operating procedures for infant diagnosis
  - Establish linkage between testing and immunization visits

- Establish linkages between the different health centers for the mother and infant (PMTCT programs, child health services, ART programs etc.)
- Operationalize the use of dried blood spots
- Develop algorithms for early infant testing, including when infants should be tested
- Examine how to reduce the loss to follow up rate
- Enhance counseling materials, especially for stigma and disclosure issues
- Develop a mechanism that allows the healthcare worker to know if the child has been exposed to HIV
- Develop projections and estimations of short and long term needs for procurement

- Training, capacity building, laboratory set-up
  - Develop training manuals for the different testing technologies
  - Establish external quality assurance programs
  - Establish guidelines on what National Reference Laboratories need to have in place to demonstrate that they can perform quality PCR for infant diagnosis
  - Expand laboratory capacity and the training of healthcare workers to meet the demands of the rollout of infant diagnosis programs
  - Evaluate mechanisms to reduce the turnaround time between drawing the sample and receiving the test result
  - Introduce independent validation of performance for the different test platforms (this should include testing in the conditions where it will be used)

Workshop participants discussed the next steps for the Forum working group on infant diagnosis.

The following priority areas were identified:

- CD4/CD8 ratio (a working group was formed at the meeting to try and validate the role of CD4/CD8 ratio for early infant diagnosis using data from the US as well as international cohorts)

- Compilation of data available on normative values across populations

- Working group to develop consensus on virologic questions:
  - Clarify the role of rapid testing and develop recommendations for its implementation +/- breastfeeding
  - Discuss issues associated with pre-exposure to PMTCT interventions and their possible impact on subsequent treatment of children
  - Discuss integration of ongoing serologic testing programs with virologic approaches
  - Identify ways to capture the experience from different programs and sponsors to inform guidelines and policy; inform what is programmatically efficient
  - Develop consensus on p24 antigen assay: is there a need to keep this going, and if so, who will champion it?
  - Maintain the networking role of the Forum infant diagnostic group to provide partnership and discussion platforms beyond individual programs; provide a bridging mechanism between research and programmatic activities
  - Critically review the currently available PCR data
  - Develop a summary table indicating current technologies, summarizing level of experience, appropriateness for different settings

APPENDIX A

Strength of recommendation	Level of evidence to guide recommendation
A. Recommended − should be followed B. Consider − applicable in most situations C. Optional	I. At least one randomized controlled trial with clinical endpoints or several relevant[a] high-quality descriptive or observational studies. II. At least one randomized controlled trial with surrogate markers, at least one high-quality study or several adequate studies. III. Observational cohort data, one or more case-controlled or analytical studies adequately conducted. IV. Expert opinion based on evaluation of other site or program experience.

[a] Refers to studies that are representative of age, sex, genetic background and geography.
Source: Adapted from references (Briss, Zaza et al. 2000; 2005), and:
WHO Evidence Network, http://www.euro.who.int/HEN/Syntheses/hepatitisC/20050408_5
Evidence-based medicine, http://ebm.bmjjournals.com/
US Preventive Service Task Force, http://www.ahrq.gov/clinic/epcix.htm

TABLE 1

Intervention	Current Coverage	Number in need in 2010	Costs in US$ (through 2008)
Care and support for OVC	15%*	19.7 million	6 Billion
PMTCT (prong 3)	3%	2.9 million	800 million
Cotrimoxazole prophylaxis	1%	5.1 million	
ART for children	2%	1.2 million	
All prevention			*29 billion*
VCT	1%	51.5 million	1.7 billion
Harm Reduction	4%	7.2 million	440 million
SW interventions	16%	17.6 million	1.6 billion
MSM interventions	11%	21.8 million	1.2 billion
Youth in school	50%	122 million	313 million
Youth out of school	<10%?	145 million	2.8 billion

TABLE 2:

2005 estimates	Child (0-14 years) deaths due to AIDS	Children (0-14 years) in need of ART	Children (0-18 months) in need of ART	Children (0-14 years) in need of cotrimoxazole - diagnosis at 18 months	Children (0-14 years) in need of cotrimoxazole - diagnosis before 18 months
Global	**410,000**	**660,000**	**270,000**	**4,000,000**	**2,100,000**
Caribbean	**3,100**	**5,100**	**1,800**	**29,000**	**15,000**
East Asia	**1,500**	**1,900**	**1,700**	**17,000**	**7,600**
Eastern Europe & Central Asia	**1,100**	**1,600**	**1,100**	**18,000**	**6,200**
Latin America	**6,000**	**8,600**	**400**	**70,000**	**35,000b**
North Africa & Middle East	**5,300**	**7,600**	**4,400**	**59,000**	**18,000**
Oceania	**<500**	**<500**	**<500**	**2,000**	**<1000**
South & South East Asia	**26,000**	**37,000**	**21,000**	**290,000**	**130,000**
Sub-Saharan Africa	**370,000**	**600,000**	**240,000**	**3,500,000**	**1,900,000**
PEPFAR countries	**250,000**	**410,000**	**200,000**	**2,400,000**	**1,300,000**
Asia	**28,000**	**39000**	**23000**	**310,000**	**140,000**
Latin America & Caribbean	**9,200**	**14,000**	**5,800**	**100,000**	**50,000**

TABLE 3

Method of diagnosis	Recommendations for use	Strength of recommendation/ level of evidence*
Virological methods (Includes DNA, RNA, and ultrasensitive p24)	To diagnose infection in infants under age 18 months; initial testing is recommended at age 6-8 weeks	**A (I)**
HIV Antibody testing	To diagnose HIV infection in mother or identify HIV exposure of infant	**A (I)**
HIV Antibody testing	To diagnose HIV infection in children 18 months or older	**A (I)**
HIV Antibody testing	To identify HIV positive children under 18 months in whom HIV infection is likely[a]	**A (IV)**

Notes:

a. Children less than 18 months of age who have reactive HIV antibody tests include children who are truly HIV-infected, as well as those who still have maternal antibody but are uninfected. By the age of 12 months most HIV infected children have signs and symptoms associated with HIV infection and if these children are HIV antibody positive, then they are likely to be HIV infected.

* The strength of grading of recommendations and levels of evidence can be found in Appendix A

FIGURE 1

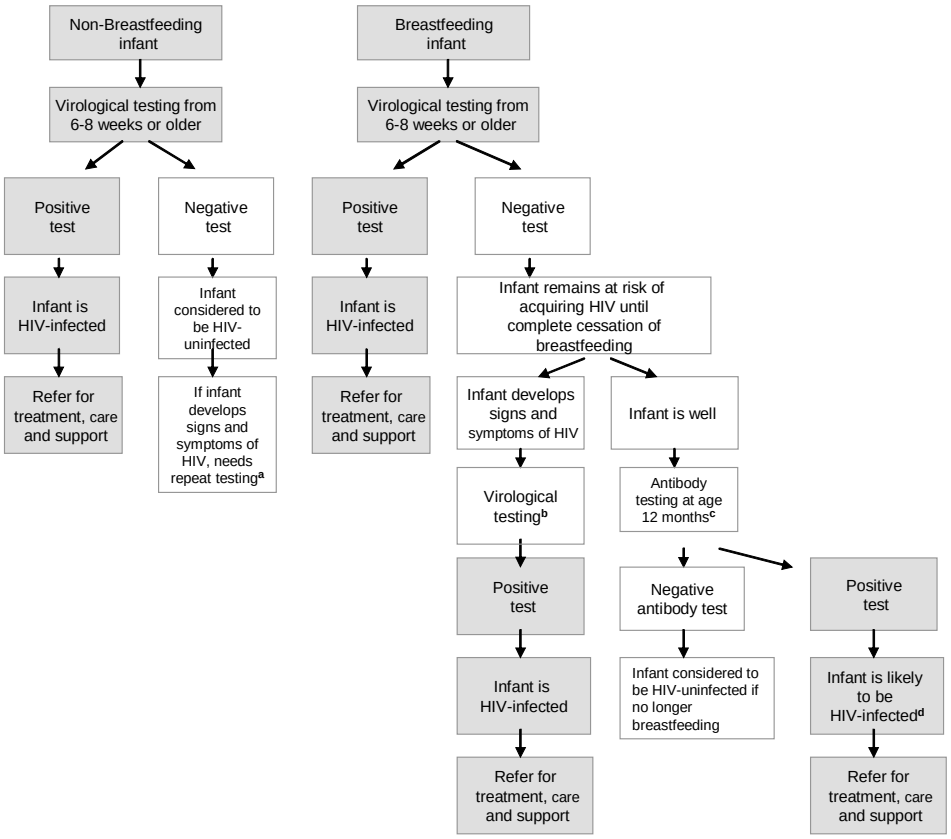
Non-Breastfeeding infant
Virological testing from 6-8 weeks or older
Positive test
Negative test
Infant is HIV-infected
Infant considered to be HIV-uninfected
Refer for treatment, care and support
If infant develops signs and symptoms of HIV, needs repeat testing[a]
Breastfeeding infant
Virological testing from 6-8 weeks or older
Positive test
Negative test
Infant is HIV-infected
Infant remains at risk of acquiring HIV until complete cessation of breastfeeding
Refer for treatment, care and support
Infant develops signs and symptoms of HIV
Infant is well
Virological testing[b]
Antibody testing at age 12 months[c]
Positive test
Negative antibody test
Positive test
Infant is HIV-infected
Infant considered to be HIV-uninfected if no longer breastfeeding
Infant is likely to be HIV-infected[d]
Refer for treatment, care and support
Refer for treatment, care and support

TABLE 4

. Laboratory parameters for monitoring infants and children at baseline, prior to ART and during ART					
Diagnosis and monitoring laboratory tests		**Baseline**	**Monthly at initiation of 1st or 2nd line regimen (weeks 4, 8, 12)**	**Every 6 months**	**As required (i.e., symptom-directed)**
HIV diagnostic testing: virological and Ab		✓	-	-	-
Haemoglobin [a]		✓	✓	-	✓
WBC and differential		✓	✓	-	✓
%CD4 or Absolute CD4 cell count[b]		✓	-	✓	-
Pregnancy testing in adolescent girls[c]		✓	-	-	-
Full chemistry (including, but not restricted to, ALT[d], liver enzymes, renal function, glucose, lipids, amylase, lipase, and serum electrolytes)[e]		-	-	-	✓
Diagnostic tests for treatable co-infections and major HIV/AIDS-related opportunistic diseases	Screening for TB and malaria (basic microscopy; i.e. sputum smear test for TB and thick blood drop smear test for malaria diagnosis)[f]	-	-	-	✓
	Full cerebrospinal fluid (CSF) microscopy (including India ink for cryptococcal meningitis), in adolescents: syphilis and other STI diagnostic tests.	-	-	-	✓
	Diagnostic tests for hepatitis B, hepatitis C serology, bacterial microbiology, and cultures and diagnostic tests and procedures for PCP, *Cryptococcus*, toxoplasmosis and other major OIs)	-	-	-	✓
HIV viral load measurement [g]		-	-	-	✓

TABLE 5

Reference Center	Nucleic Acid Testing (DNA/RNA) Expensive Complex Technology Considered Gold Standard
Provincial or District Level	Ultrasensitive p24 Antigen/Reverse Transcriptase assay? Lower Cost Less Complex Technology
Primary Care or Rural Setting	Ship Samples (Dried Blood Spots or Fixatives) Least Resource Intensive Least Complex

TABLE 6

Time from Birth	N	Sensitivity	Specificity
0-7 days	114	62.7%^ 94.7%*	99.7^ 99.0%*
8-30 days	180	91.6%^ 93.8%*	98.4^ 99.1%*
31-90 days	368	94.4%^ 96.1%*	97.6%^ 98.6%*
91-180 days	141	91.4%^ 94.0%*	98.4%^ 98.6%*
>180 days	93	97.0%^ 94.0%*	95.4%^ 99.1%*

^ kit buffer * external buffer

TABLE 7

Author	N	Buffer	% Sensitivity	% Specificity	Subtype
Sutthent	142	BioMer	100	100	A/E, B
Sherman	203	Kit	98	98.5	C
Nouhin	167	Kit?	91.1	99.2	A/E
DeBaets	150	Ext	92.3	100	multiple
DeBaets	87	Ext	100	100	multiple
Zijenah	164	Kit	96.7	96.1	C
Patton	141	Ext	98.8	100	C
Respess	757/482	Kit/Ext	93/98.4	95.6/98.9	B

TABLE 8

	PCR Positive (n = 76)	PCR Negative (n = 61)	*P* value[a]
Median age (months)	5.5 (IQR: 3–13)	8 (IQR: 4–14)	0.08
Median $CD4^+$(cells/μL)	521.5 (IQR: 323–805)	1356 (IQR: 916–1769)	<0.001
Median $CD8^+$(cells/μL)	1302.5 (IQR: 829–2054)	799 (IQR: 471–1020)	<0.001
Median CD4/CD8 ratio	0.4 (IQR: 0.3–0.6)	1.8 (IQR: 1.4–2.3)	<0.001
Median %CD4	13.9 (IQR: 9.2–19.1)	29.9 (IQR: 25.3–34.5)	<0.001
Median %CD8	31.3 (IQR: 22.3–42.9)	18.4 (IQR: 14.2–21.5)	<0.001

Abbreviations: PCR, polymerase chain reaction; n, number tested; *P* value[a] for statistical significance between group medians was estimated using the Kruskal-Wallis test.

TABLE 9

	0–18 months infant age group (n = 137)	0–11 months infant age group (n = 95)	12–18 months infant age group (n = 42)
%Sensitivity	98.7 (CI: 96.1–100)	98.2 (CI: 94.7–100)	100 (CI: 100–100)
%Specificity	98.4 (CI: 95.2–100)	97.5 (CI: 92.7–100)	100 (CI: 100–100)
%PPV	96.4	94.6	100
%NPV	99.4	99.2	100
%TE	98.5 (CI: 96.5–100)	97.9 (CI: 95.0–100)	100 (CI: 100–100)

Abbreviations: n, number tested; NPV, negative predictive value; PPV, positive predictive value; TE, test efficiency

TABLE 10

Age	n	HIV pos	HIV neg	Doctor's examination	Doctor's examination	IMCI clinical algorithm	IMCI clinical algorithm
				sensitivity	specificity	sensitivity	specificity
6 weeks	301	26	275	56%	90%	17%	97%
3 months	290	23	267	67%	96%	11%	100%
7 months	258	20	238	94%	99.5%	47%	99.6%
12 months	234	15	219	93%	99.5%	50%	99.5%

TABLE 11

		HIV infected children	Sensitivity	HIV uninfected children	Specificity
Serum HIV ELISA (n=291)	+	20	100%	112	
	-	0		159	59%
Oral fluid HIV ELISA (n=291)	+	19	95%	18	
	-	1		253	93%
OraQuick (n=235)	+	13	87%	6	
	-	2		214	97%

False negative results

False positive results

FIGURE 2

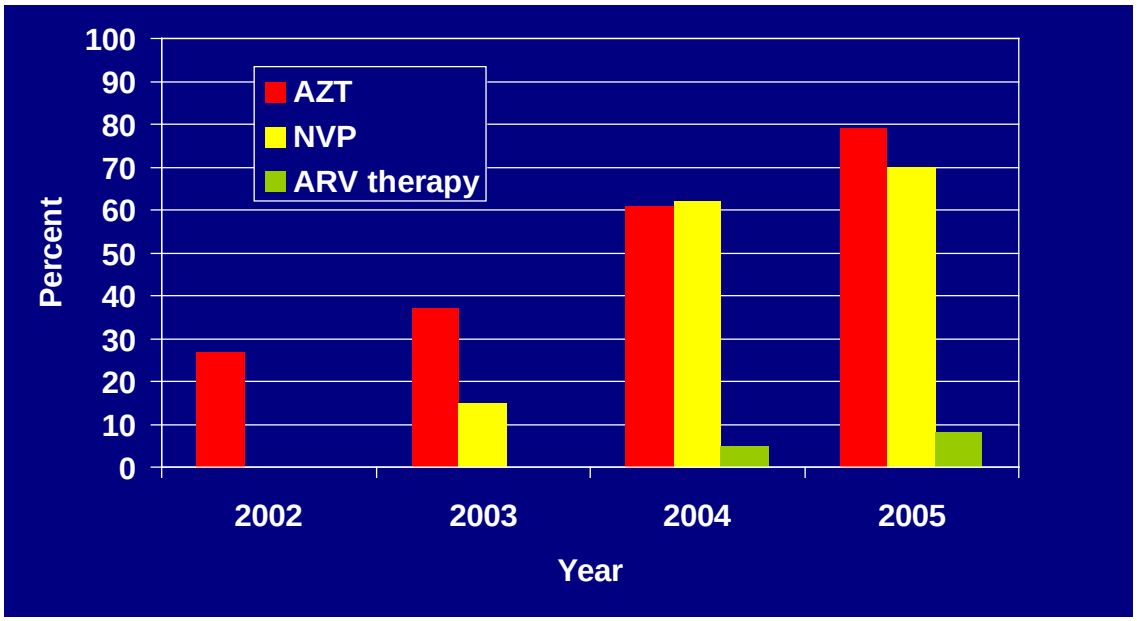
100
90
80
70
60
50
40
30
20
10
0
Percent
AZT
NVP
ARV therapy
2002
2003
2004
2005
Year

FIGURE 3

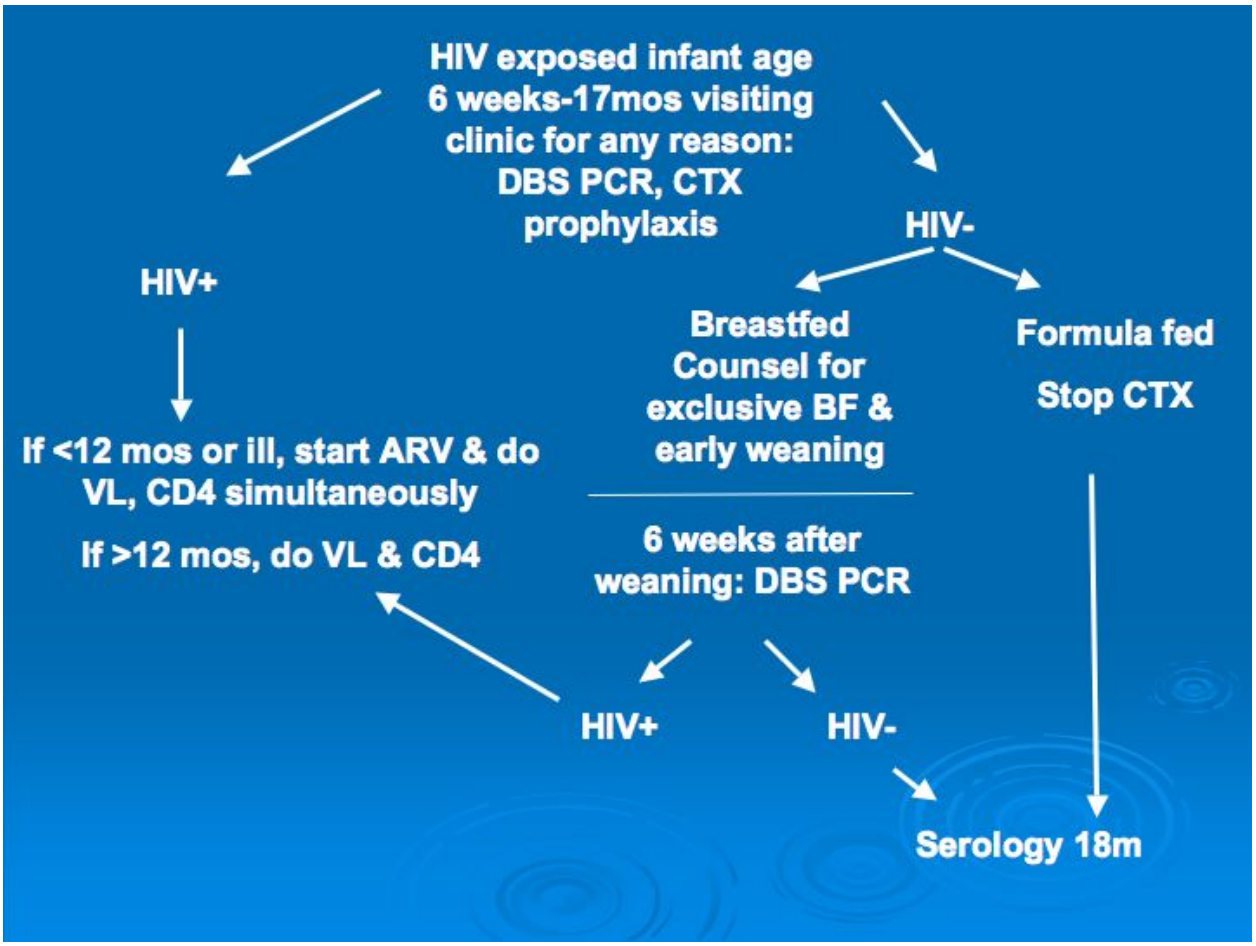
HIV exposed infant age 6 weeks-17mos visiting clinic for any reason: DBS PCR, CTX prophylaxis
HIV+
HIV-
If <12 mos or ill, start ARV & do VL, CD4 simultaneously
If >12 mos, do VL & CD4
Breastfed Counsel for exclusive BF & early weaning
Formula fed
Stop CTX
6 weeks after weaning: DBS PCR
HIV+
HIV-
Serology 18m

FIGURE 4

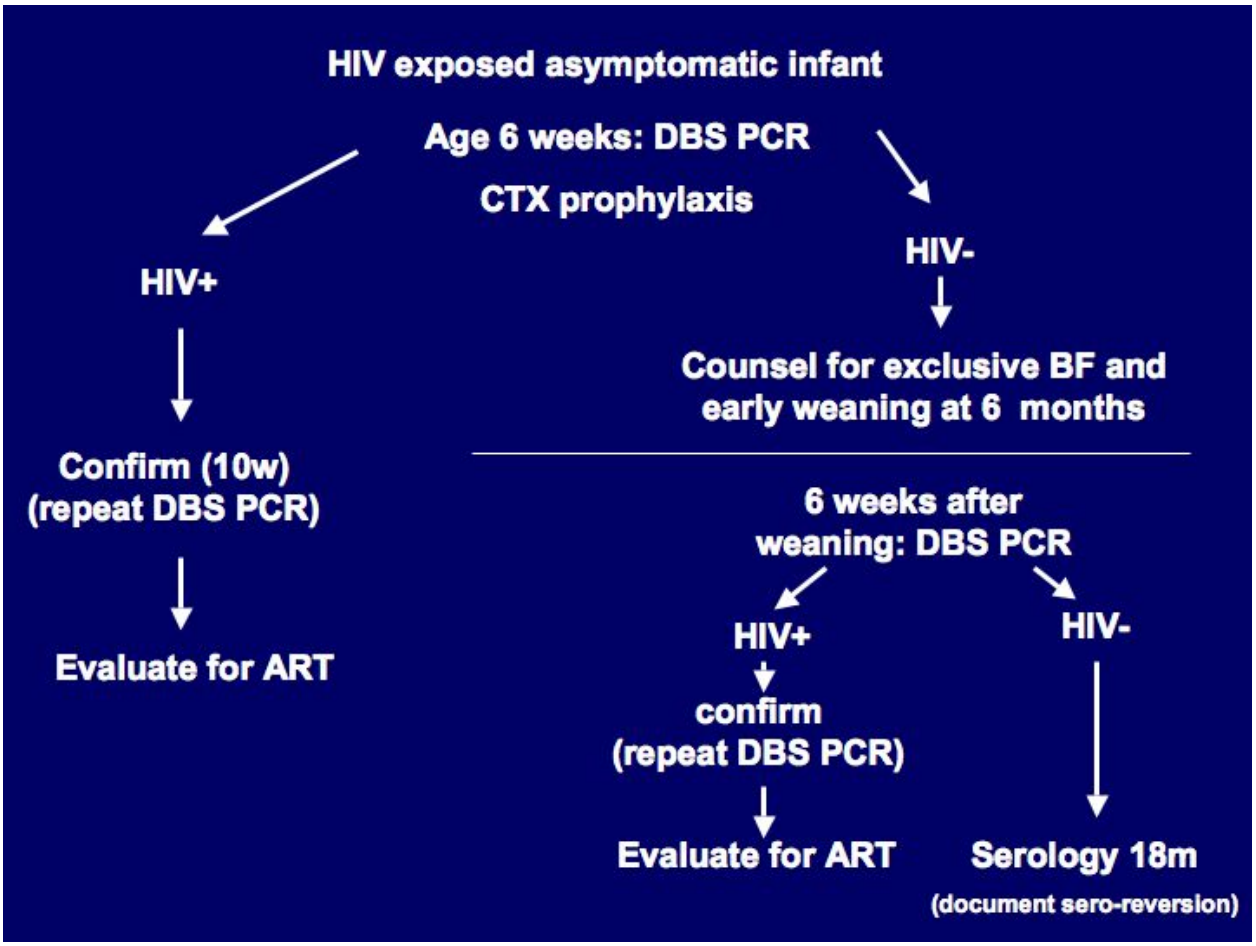
HIV exposed asymptomatic infant
Age 6 weeks: DBS PCR
CTX prophylaxis
HIV+
HIV-
Counsel for exclusive BF and early weaning at 6 months
Confirm (10w) (repeat DBS PCR)
Evaluate for ART
6 weeks after weaning: DBS PCR
HIV+
HIV-
confirm (repeat DBS PCR)
Evaluate for ART
Serology 18m
(document sero-reversion)

TABLE 12

PMTCT intervention	N	% expected positive	% actually positive
Nothing	13	35-40	31
Nothing to mother, AZT/NVP/formula for baby	22	12-20	9
AZT to mother (median 49 days, many also rec'd NVP)	1108	2-8	3.7
ARV therapy to mother	170	<1	0.7

TABLE 13

	<2 Months	2-18 Months	Total
Positive	**3 (16%)**	**17 (12%)**	**20 (12%)**
Negative	**16 (84%)**	**128 (88%)**	**144 (88%)**
Total	**19**	**145**	**164**

Pediatric Diagnosis and Monitoring Working Group Meeting

Grand Hyatt
1750 Welton St
Denver, CO 80202
Meeting Room: Grays Peak-Hyatt Conference Center (attached to Grand Hyatt)

Agenda

Aims of the Working Group Meeting are to:

1. Bring together a group of experts with interest in infant diagnosis and monitoring for use in resource limited settings

2. Provide a scientific forum to facilitate
   a. the exchange of information on infant HIV diagnosis and pediatric HIV treatment monitoring
   b. the identification of gaps in knowledge and research to address these gaps

   This will be accomplished by activities including workshops, website, quarterly updates on articles and abstracts; working group conference calls

3. Use Working Group and Forum for Collaborative HIV Research activities to help catalyze and jumpstart development of infant HIV diagnosis and antiretroviral treatment monitoring tools

4. Support and foster collaborative operational projects on infant diagnosis and monitoring for use in resource limited settings.

8:30 – 8:45	Welcome and Introductions	Mary Glenn Fowler, Tom Denny, Ben Cheng
8:45 – 9:00	Goals of the Workshop	Mary Glenn Fowler and Tom Denny
	Setting the Stage	Moderators: Mary Glenn Fowler and Tom Denny
9:00 – 9:15	The Global Situation and Special Challenges for Infant HIV Diagnosis	Robert Gass
9:15 – 9:30	WHO Testing Recommendations and Pediatric Treatment Guidelines	Siobhan Crowley
9:30 – 9:45	Policy Development in the Context of Market Projection	Tom Denny

9:45 – 10:30	Moderated Discussion: Current and projected program activities to meet infant diagnosis needs	All, with special emphasis on program and sponsor representatives
10:30 – 10:45	Break	
	State of the Art Technologies and Gaps	Moderator: John Nkengasong and Lynne Mofenson
10:45 – 10:50	Overview of Infant/Childhood Diagnosis Approaches	Emilia Rivadeneira
10:50 – 11:05	Overview of diagnosis based on HIV detection/quantification	Susan Fiscus
11:05 – 11:20	Overview of role of immunological markers in HIV diagnosis	Savita Pahwa
11:20 – 11:35	Diagnosis in clinical context	Gayle Sherman
11:35 – 11:40	Review of New Immunology Technologies from CROI	Alan Landay
11:40 – 11:45	Review of New Virology Technologies from CROI	Lisa Frenkel
11:45 – 12:30	Moderated Discussion: Where do we go from here?	All, with special emphasis on diagnostic industry representatives and clinical research network representatives
12:30 – 13:30	Lunch	
	Infant Diagnosis Program Implementation	Moderators: Nathan Shaffer and Elaine Abrams
13:30 – 13:40	Overview of PEPFAR Approach to Infant Diagnosis	Nathan Shaffer
13:40 – 14:00	Examples of Programmatic Models for Early Diagnosis	Tracy Creek and Thomas Finkbeiner
14:00 – 14:15	Operational Projects Linking Infant Diagnosis to Treatment	Dorothy Mbori-Ngacha
14:15 – 15:15	Moderated Discussion: Lessons learned and way forward	All, with special emphasis on program representatives
15:15 – 15:30	Break	
15:30 – 17:00	Next Steps: Re-cap; working group formation & assignments; publications, etc	Mary Glenn Fowler, Tom Denny, Ben Cheng

APPENDIX C: PARTICIPANT LIST

Forum for Collaborative HIV Research

"Pediatric Diagnosis and Monitoring Working Group Meeting"

February 9, 2006: Grand Hyatt; Denver, CO

Elaine Abrams Columbia University	**Robert Gass** UNICEF
Arlene Bardeguez University of Medicine & Dentistry-New Jersey	**Amy Ginsburg** The Elizabeth Glaser Pediatric AIDS Foundation
Bernard Branson CDC	**Ed Handelsman** National Institutes of Health (NIH)/NIAID
Omotayo Bolu CDC	**Marcia Kalish** CDC
Jim Bremer Rush University Medical College	**Alan Landay** Rush University Medical College
Jeanne Brosnan BD Biosciences	**Veronica Miller** Forum for Collaborative HIV Research
Mark Bulterys CDC Zambia	**Lynne Mofenson** National Institutes of Health (NIH)
Ben Cheng Forum for Collaborative HIV Research	**Dorothy Mbori Ngacha** CDC
Polly Clayden HIV i-Base	**John Nkengasong** CDC
Mark Cotton University of Stellenbosch	**David Olson** Médecins Sans Frontières (MSF)
Tracy Creek CDC	**Savita Pahwa** University of Miami Medical School
Siobhan Crowley WHO	**Jennifer Read** National Institutes of Health (NIH)
Halima Dao CDC	**Renee Ridzon** The Bill & Melinda Gates Foundation
Michel De Baar Primagen	**Emilia Rivadeneira** CDC
Felipe de la Vega Médecins Sans Frontières (MSF)	**Bill Rodriguez** Harvard Medical School & Clinton Foundation
Thomas Denny University of Medicine & Dentistry-New Jersey	**Jeff Safrit** The Elizabeth Glaser Pediatric AIDS Foundation
Ken Dominguez CDC	**Nathan Shaffer** CDC
Gary Douglas LabNow Inc.	**Gayle Sherman** University of Witwatersrand in Johannesburg

Thomas Finkbeiner CDC	**Andrea Swartzendruber** CDC
Susan Fiscus University of North Carolina-Chapel Hill	**Vicki Tepper** University of Maryland School of Medicine
Joe Fitzgibbon DHHS/NIH/NIAID/DAIDS/TRP/DDCSB	**Angela Vernon** Beckman Coulter
Mary Glenn Fowler Makerere University-JHU	**Cathy Wilfert** The Elizabeth Glaser Pediatric AIDS Foundation
Kim Fox Thailand MOPH-U.S. CDC Collaboration	**Carol Worrell** HIV Research Branch, TRP, DAIDS
Lisa Frenkel University of Washington	

APPENDIX D: ABBREVIATIONS AND ACRONYMS

AIDS	acquired immunodeficiency syndrome
ANC	antenatal care
ART	antiretroviral therapy
ARV	antiretroviral (drugs)
AZT	zidovudine (Retrovir)
CDC	Centers for Disease Control and Prevention
CDC-GAP	Centers for Disease Control and Prevention – Global AIDS Program
DBS	dried blood spots
DNA	deoxyribose nucleic acid
HIV	human immunodeficiency virus
EIA	enzyme immunoassay (also known as enzyme linked immunosorbent assay [ELISA]
HLA	human leukocyte antigen
ICD	immune complex dissociated
IgA	immune globulin A
IgM	immune globulin M
IMCI	integrated management of childhood illness strategy
MSM	men who have sex with men
MTCT	mother-to-child transmission
NASBA	nucleic acid sequence-based amplification
NIH	National Institutes of Health
OVC	orphans and vulnerable children
PCP	pneumocystis carinii pneumonia
PCR	polymerase chain reaction
PEPFAR	President's Emergency Plan for AIDS Relief
PMTCT	prevention of mother-to-child transmission
p24	p24 antigen
RNA	ribonucleic acid
SBIR	Small Business Innovation Research
SW	sex worker
TB	tuberculosis
VCT	voluntary counseling and testing
WHO	World Health Organization

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