

# HATiP

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# Early infant HIV diagnosis

## By Theo Smart

This HATIP reviews discussions at the 2007 Implementers' Meeting and at the International AIDS Society (IAS) meeting a month later on early infant diagnosis, as well as some of the recent medical literature touching on the subject.

## With thanks to:

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## Key points

The first and best way to promote good health outcomes in HIV-exposed children is to make certain that every pregnant woman at risk of HIV receives a diagnosis.

- If she is negative, she needs counselling and support to keep her that way since a woman is at high risk of infection during and just after pregnancy — and if infected, she is much more likely to transmit HIV to her baby.
- If she is positive, she should receive:
  - the best care and treatment for her own health (including antiretroviral therapy (ART) for those who need it);
  - if she does not need ART for her own health, an optimal regimen to prevent-mother-to-child transmission (PMTCT) should be prescribed.

## Universal access to these HIV testing, counselling, care and prevention services should keep the vast majority of HIV-exposed children uninfected.

In order to provide universal access, these HIV-related interventions must be more closely integrated with strengthened antenatal and other maternal child health (MCH) services to deliver the essential healthcare services needed by all mothers and infants in resource-limited settings.

But at present, there are more than 2 million children with HIV, most of whom did not receive adequate PMTCT in time, and many of these children and their mothers remain undiagnosed.

## Initiating ART in HIV-infected infants as early as 6-8 weeks (as soon as their infection status can be determined) dramatically reduces their risk of early death. In addition, identification and treatment of HIV-infected mothers can improve the survival of both the mother and her children.

Early infant diagnosis presents logistical challenges and is costly but possible

- Antibody tests are unreliable in young infants while they carry their mother's antibodies (out to 18 months though most infants will lose maternal antibodies long before that. WHO recommends rapid testing for infants >9 months old, followed by PCR only for those who are still antibody-positive.

- Drawing, processing and shipping liquid blood samples from babies for PCR testing is impractical.

## However, dried blood spots (DBS) can easily be collected from infants, packaged and sent to a centralised lab for reliable HIV testing by DNA PCR (see box 1 & 2 for procedure)

HIV DNA PCR requires somewhat different equipment and techniques from those presently available in the labs scaling up viral load (HIV RNA) capacity in resource-limited settings — which raises the issue of how laboratory capacity should be allocated.

- Running viral load tests on DBS has potential, but may not be as reliable or sensitive in infants whose mothers are on ART or who have received complex PMTCT regimens. Nevertheless, there would be significant advantages to using the same technology which is already being rolled out in many settings, so further research is *urgently* needed to validate the clinical utility of performing viral load on DBS samples from ART or PMTCT-exposed infants.
- Other technologies are in development, but a point-of care test which could provide results on the spot seems a long way off.

For now, HIV DNA PCR on DBS is the gold standard for early infant diagnosis, but since this capacity will be very limited in most countries, the pilot programmes described in this article focus on setting up reliable and timely ways to transport DBS to centralised labs and to return the results as quickly as possible and get those infants who are positive into care and on ART

Testing remains expensive but using a rapid HIV antibody test (RHT) to screen out negative results could reduce the number of samples that need to be collected and sent for PCR (see algorithm in article).

DBS should be performed on all HIV-exposed children, preferably at the time when they attend their first immunisation clinic.

HIV testing and counselling should be expanded in the mother-and-child health (MCH) systems of high HIV burden countries to identify all the mothers with HIV and HIV-exposed children, perhaps starting in the sick baby clinics since they will contain the most babies with HIV.

Community-based approaches are needed to expand access and acceptance of HIV testing and counselling for mothers and infants.

Adequate training, improved tracking of patients and their samples, timely transport of specimens, turn-around in the lab and delivery of results is crucial for DBS — otherwise, it is a waste of time.

For DBS to work, centralised referral laboratories will need to be strengthened to take on these new tasks and prevent backlogs. A long turn-around time could mean a useless result (and a child's death).

Counselling has to be adapted to meet the challenges of giving parents what could be confusing test results.

## There is a serious danger that mothers with HIV who receive an HIV-negative result on their infant will wean the child prematurely. The risk of early weaning and subsequent death of negative babies may be significant if the implications are not well understood by health providers and mothers.

Exclusive breastfeeding counselling must be reinforced, and programmes should consider ways of making breastfeeding safer, such as putting mothers at risk of transmitting HIV onto ART.

## GET HIV-positive children quickly into care and treatment, otherwise testing is pointless

Newer, cheaper, faster tests for infants with HIV are urgently needed.

## Putting systems in place for early infant diagnosis and treatment

Treating HIV-infected infants with antiretroviral therapy (ART) as soon as possible —within the first six to 12 weeks of life —reduces early mortality by 75%, according to the results from the Children with HIV Early Antiretroviral Therapy (CHER) trial presented at the 4th International AIDS Society Conference on HIV Treatment and Pathogenesis in Sydney Australia last year (Violari) (for a more complete review of the results, see <http://www.aidsmap.com/en/news/973ABB13-4482-4A06-92BC-C3298E9EF6E.asp>).

Since more than half of the children who are HIV-infected may die before reaching two years of age without ART, and the median age of death in those who die early is around 6 months (Newell), these findings provide an obvious incentive, if not a moral obligation, to locate HIV-exposed infants, test them for HIV infection as soon as possible, and to get these children and their families into care and/or onto treatment.

Early infant diagnosis can serve as the bridge between prevention, care and treatment — and gauge the effectiveness of programmes for the prevention of mother to child transmission (PMTCT). But until recently, learning an infant's HIV status at an early age has not been seen as financially or logistically feasible in most resource-limited settings. Recent programmatic experience with systems incorporating the collection of dried blood spots (DBS), which can be easily performed at even the most remote clinics and sent to a centralised laboratory for HIV testing (by PCR), may change all that.

"DBS is a simple procedure — as simple as doing a malaria slide — it's not very difficult," said Dr. Mildred Mudany of the CDC in Kenya at last year's HIV Implementer's Meeting in Kigali, Rwanda, where reports about the implementation of DBS/HIV PCR systems for early infant diagnosis were made by nine PEPFAR-focus countries.

However, while the procedure itself is fairly simple, how it is introduced into public health systems poses its own set of operational challenges — especially in contexts where antenatal care, prevention of mother to child transmission programmes, and maternal and child health systems are not well-integrated, other basic maternal-child health interventions are not being implemented, reference laboratories are poorly equipped and staffed, systems for the timely delivery of samples and results aren't established, and linkages to HIV care and treatment are weak.

These services must be strengthened in tandem and supported by community-based care systems for better case-detection and follow-up —to make certain that HIV-exposed children (and their mothers) don't fall through the cracks and do get linked into care.

## Background on HIV testing in infants

Conventional antibody testing cannot reliably detect HIV infection in young children, since maternal antibodies to HIV can be passed onto the infant through the womb or through breast milk. These maternal antibodies may continue to be detected in uninfected infants out to 18 months using standard ELISA tests, though most infants will lose maternal antibodies long before that. WHO recommends rapid testing for infants >9 months, followed by PCR only for those who are still antibody-positive (see <http://www.aidsmap.com/en/news/DAEE0A6A-109A-4D04-81C8-D>

[88E111E0991.asp](http://www.aidsmap.com/en/news/DAEE0A6A-109A-4D04-81C8-D) and more on this below). Nevertheless, study after study report the majority of HIV-exposed children are lost to follow-up long before that — and that, if infants cannot be tested earlier, the opportunity to get them into care and treatment could be lost.

Directly testing for HIV using PCR, on the other hand, is accurate when performed within 6 weeks of the last exposure (Dunn). But the test itself is expensive and can only be conducted at very well equipped laboratories by highly trained staff. But perhaps the greatest problem has been the logistics at the local clinic level: drawing adequate liquid blood samples from infants is difficult for non-specialists, and the proper processing, storage and transporting of so many liquid samples is simply too complex in many remote resource-limited settings.

## DBS testing: accurate and more practical

"Dried blood spots (DBS) can overcome the blood sampling and logistical obstacles that limit access to infant diagnosis in low-resource settings," Dr Gayle Sherman and colleagues in Johannesburg wrote in *JAIDS* in 2005. Their prospective cohort study comparing PCR testing on DBS versus liquid blood specimens from 288 infants at the age of 6 weeks, yielded an accurate diagnosis of HIV infection status, with only one false positive, which was detected on repeat testing.

A large-scale evaluation project, by Dr Tracy Creek and colleagues in Botswana, successfully piloted the use of DBS HIV PCR as part of routine infant care in a public health system. A preliminary account of Dr Creek's results were first presented at the 2006 HIV Implementers meeting in Durban, South Africa, but has since been published in the January 1<sup>st</sup> 2008 issue of the *Pediatric Infectious Disease Journal*.

This joint project between Botswana and the CDC developed new policies, procedures, and training materials to introduce a DBS testing system into 15 clinics and one referral hospital in Francistown, and the Botswana-Baylor Children's Center of Excellence Clinic in Gaborone.

The collection, drying and packaging procedures were relatively simple. Drops of blood from each infant were taken from a toe or heel prick by the local clinic staff on filter paper, then dried, packed with desiccant and stored and/or sent to the centralised laboratory (For a more detailed description of the procedure, and related resources, see boxes 1 and 2).

Training on the procedure consisted of a one-day classroom session given to clinic staff, followed by a few days practical supervised training on-site. Then, over a six-month period between June and December 2005, staff collected and shipped specimens from 1931 HIV-exposed infants to a centralised laboratory for testing. The laboratory only rejected 27 (1.4%) samples — mostly due to labelling errors. 136 (7.0%) of all the infants were HIV-infected and entered into care. Retesting of 150 random samples for quality assurance at US Centers for Disease Control (CDC) in Atlanta showed 100% concordance with the tests performed in Botswana.

After this successful project, the Botswana Ministry of Health and CDC gave the go-ahead to expand DBS collection nationwide during 2006–2007 with the goal of providing "early diagnostic services to the approximately 13,300 HIV-exposed infants born in Botswana each year" (more on the challenges scaling up to that level in Botswana is reported below).

Further experiences using DBS for PCR testing in Rwanda, Mozambique and South Africa were then presented at the World AIDS Conference in Toronto in 2006.

Dr Luis Manuel Felipe Gonzalez, who works as laboratory adviser for the International Center for AIDS Care and Treatment Programs (ICAP) in Eastern Africa, reported on the introduction of DBS in Rwanda — and described how the logistics (transport, follow-up, etc.) were adapted somewhat to meet the local needs and resources in that environment (this will be discussed in more detail below).

The lab and sites Dr Gonzalez set up also served as centres of excellence, training those interested in introducing similar programmes into neighbouring countries.

Mozambique also piloted a project, reporting that DBS “allowed the diagnosis of children from remote areas... and serves as a tool for treatment and care entry, establishment of follow-up programs for both HIV-positive and breast-feeding HIV-negative infants, implementation of guidelines for counselling on infant feeding, and allows treatment of exposed children in a timely manner.” (BilaRamalho).

Dr Nigel Rollins of the University of KwaZulu-Natal, reported on the use of DBS in a pilot programme set up to perform routine anonymous HIV surveillance testing on all infants aged between four to eight weeks who were brought in for their first DTP immunisation at seven primary health care clinics offering PMTCT services in KwaZulu Natal (KZN).

The protocol was different: DBS were collected from 2,439 infants between the ages of 4-8 weeks and first screened for antibodies to determine whether the infant was HIV-exposed, and then, the 907 samples that were antibody-positive were tested for HIV by PCR. The study detected a vertical transmission rate of 20.8% in 6-week old infants of HIV-positive mothers — and served as a clear indication that the PMTCT programme in that province was not reaching its targets (for more on this study, see <http://www.aidsmap.com/en/news/74A5B372-0FAF-4EB1-8825-F84DDAB7D367.asp>).

Dr. Rollins said the procedure “has the added advantage not only of identifying children early for treatment — getting them and the mother as well into the continuum of care, but it is a very effective way of monitoring the PMTCT system.”

## On the proper collection, drying and packaging of DBS

### Materials from Botswana

*The following is text derived from two training materials posters put together by Tracy Creek and colleagues at the CDC for the Francistown pilot study*

### Collection of Dried Blood Samples from Infants for PCR testing

#### 1. Gather necessary supplies

- Gloves
- Blood collection card (filter paper)
- Lancet (2mm) (the Botswana study used a self-springing lancet)
- 70% spirit or alcohol

- Gauze or cotton wool
- A pen

#### 2. Complete all necessary paperwork

- Infant diagnosis registration form
- Clinic register
- Laboratory request/report form
- DBS card

#### 3. Choose the area to be pricked and ask the mother to warm this area

- Infants 6 weeks-4 months: heel
- Infants 4 months -10 month: big toe
- Infants >10 months or >10 kg: finger

#### 4. Wash and glove hands. If gloves have powder, wash off powder

#### 5. Position the baby with the foot or hand down, then clean the spot to be pricked with spirit or alcohol, and allow to dry for 30 seconds

#### 6. Gently squeeze and release the area to be pricked until it is ready to be bled, then prick the infant in the selected spot with the 2mm lancet

#### 7. Wipe away the first spot of blood, then allow a large drop of blood to collect

#### 8. Touch the filter paper gently against the large drop and allow it to completely fill the circle. You may add new drops on top of wet drops if the circle is not full. Collect at least 3 good circles. Do not put a fresh drop on top of one that has already dried.

#### 9. Clean area, no bandage is needed.

### Drying and Packaging Dried Blood Spot (DBS) Samples

#### Drying

1. Leave DBS on a drying rack in a clean, dry, protected area for at least 4 hours or overnight.
2. Keep lab request forms with DBS cards

#### Packaging

#### 1. Wrap the individual DBS card with a glassine paper so that DBS cards will not have direct contact with each other. Insert up to 10 wrapped cards into a special sealable plastic bag.

#### 2. Add 10 desiccant packets to each bag.

#### 3 Add at least one humidity card per bag. Gently press the bag to remove most of the air before sealing.

#### 4. Use the specimen delivery checklist to check you have a lab form for each DBS.

- Place the bag of DBS, all the DBS DNA PCR lab forms and the specimen delivery checklist into a large envelope.
- Label the envelope with:



- o Name of collection site (clinic)**
- o Name of person delivering specimen**
- o Date you are sending the samples**

- Place the envelope in designated area to be picked up for the laboratory**

**For copies of these two posters (which contain full color pictures illustrating the procedure) and other related CDC materials, please contact Dr Tracy Creek at [tgc0@CDC.GOV](mailto:tgc0@CDC.GOV)**

**Materials from the International Center for AIDS Care and Treatment Programs (ICAP)**

**ICAP has also developed an extensive library of paediatric HIV/AIDS resources for clinicians in resource-limited settings: see**

**<http://www.columbia-icap.org/resources/peds/index.html>**

**One of these is a clinical manual on infant diagnosis of HIV:**

**<http://www.columbia-icap.org/resources/peds/files/Infantdx050307.pdf>**

**ICAP has also put together a ppt presentation, which also provides a detailed step by step visual demonstration on proper DBS collection, drying and packaging:**

**<http://www.columbia-icap.org/resources/peds/files/Module7-Collection,storage%20and%20transportation%20of%20DBS-2007.ppt>**

**(This is a large file, at approximately 10 MB)**

## Scaling up early infant diagnosis

These reports served as models for other countries introducing early infant diagnosis into their own programmes. By the time of last year's HIV Implementers' meeting in Kigali, Rwanda, teams from 9 countries reported on piloting DBS or gave updates on the challenges expanding from pilot studies to larger or national programmes. (See references at the end plus copies of some of the Power Point presentations can be found online here:

<http://www.hivimplementers.com/agenda/Abstracts-Agenda-Day3.html>.)

Together the programmes, represented by the panelists at the breakout session, had collected DBS samples from over 20,000 infants within the past year, up from only a couple of thousand or so reported at the meeting the previous year — leading to the diagnosis of about 2000 HIV-infected children.

The presentations also demonstrated strengths and weakness in the countries' PMTCT programmes. For instance, Dr. Dominic Karanja of Pathfinder International in Kenya reported on a pilot DBS study that found an overall infection rate of 16.6% for the sites involved — “but in some centres, the infection rate was zero, and in others it was high, around 30%! So we really need to go back and try

to see what the reason is for this variation in infection rates,” he said.

These projects didn't always run smoothly; nevertheless, on the whole, presenters and participants in the session seemed enthusiastic about the prospects of dramatically improving early infant diagnosis in their countries within the next few years.

Yet some significant challenges to full scale-up and performance remain, though the severity of the problem or the type of solution varies from country to country.

## Cost

While DBS collection may remove some of the logistical constraints to early infant diagnosis, the cost is still high.

Most of the studies described below utilise the Roche Amplicor HIV DNA test, which is a qualitative test that does not give a viral load result but rather a yes or no answer to whether HIV DNA is present in the sample. This requires the use of different laboratory equipment that is not yet available in many countries, with the requirements for additional training of staff, different reagents, and backup service.

Many programmes and policy makers are interested in whether they could rather perform viral load tests on DBS for infant diagnosis using existing equipment. Running viral load tests on DBS may have potential, but such tests may only diagnose children with detectable viral loads and thus may not be as reliable or sensitive as HIV DNA PCR in infants whose mothers are on ART or who have received complex PMTCT regimens.

Nevertheless, there would be significant advantages to using the same technology which is already being rolled out in many settings, so further research is urgently needed to validate the clinical utility of performing viral load tests on DBS samples, especially from ART or PMTCT-exposed infants.

Some newer PCR equipment, such as the Cobas Taqman, has the capacity to do both HIV RNA and DNA on liquid samples, but the extraction technique on DBS that Dr Sherman and colleagues perfected has not yet been adapted for use with this equipment. However, studies on DBS are underway and look promising for the future.

Alternative and newer technologies that may one day further reduce costs are discussed toward the end of the article. For now, the DNA test remains the gold standard test for early infant diagnosis.

In addition to the equipment costs (which Dr Gozalez estimated to be around \$50,000 in 2006), the total cost of performing each DBS test ranges roughly from \$15-21 (including the cost of using the \$8.00 Roche Amplicor HIV DNA PCR kit) depending on the lab. The cost is greater if a second test is run to confirm a positive result — but since false positives are rare, some programmes forgo this step in favor of performing an antibody test once maternal antibodies should have cleared (more below). In Botswana, the policy is to simply run a viral load test in any infant who tests positive on HIV DNA as a way to both confirm the result and stage the infant's disease and risk of progression.

## Rapid HIV antibody testing

One way to possibly cut costs would be to first use a rapid HIV antibody test (RHT) (which costs about \$1.20 per test, and is less sensitive to maternal antibodies) because some infants clear their mother's antibodies faster than others. If the result comes back negative, there is no need to send out a DBS for PCR.

Dr Jaco Homsy of the CDC in Uganda reported the results of a study using rapid HIV testing (RHT) prior to DNA-PCR for early HIV diagnosis in 394 asymptomatic HIV-exposed infants between the ages of 6 weeks and 18 months in Tororo District Hospital, Uganda.

Of the 167 infants who were tested at the first immunization visit (6 weeks to 3 months), only 11 out of 167 rapid tests came back negative – and a couple of these who were still breastfeeding later became positive anyway. However, after 3 months of age, the negative predictive value (the ability for a negative test to be reliably negative) was 96%. The positive predictive value was low under 9 months of age, but after 9-12 months rose to 86% and above. This led Homsy and colleagues to devise the following testing algorithm.

### Simplified testing algorithm

#### In HIV-exposed asymptomatic infants <18 months

- RHT for all infants starting at 6 weeks

**o RHT NEG result at any age = child is HIV-negative (unless breastfeeding)**

**o RHT POS result:**

**≥9 months: - care & refer as HIV+**

**<9 months: send for PCR**

- PCR results are definitive at any age unless child is still breastfed (BF)
- **If child is still breastfeeding**, repeat RHT >6 wks after weaning
- If not tested by PCR, confirm RHT result at 18 months

“The cost saving that was incurred by using this algorithm instead of doing PCR on all infants gave us a US \$2.40 saving per infant tested – which did not account for the turnaround [which was long for PCR results], the time wasted in terms of having the mothers come back, or the transport cost for the mothers,” said Dr Homsy. “So rapid screening of all over 6 week old HIV-exposed asymptomatic infants is a time and cost-saving strategy that could substantially reduce the number of infants requiring PCR testing (up to 35% of the infants we tested in this population).”

Clearly, the savings from using RHTs would be greater when larger numbers of somewhat older infants are being tested. The median age of children being tested for many of the pilot projects discussed at the Implementer’s Meeting was generally much higher than 6-8 weeks and this will remain the case until countries are able to scale-up testing that identifies and tests most of the 6-8 week old HIV-exposed infants visiting immunisation clinics.

But in a talk at the IAS meeting about a month later, Professor Susan Fiscus of the University of North Carolina raised a note of caution about the manner in which RHTs are being marketed in resource-limited settings.

“There are over 60 rapid antibody tests that are now available made by commercial companies who want to make some money. They go from country to country saying, ‘Well why don’t you switch from the assay you’re doing now to this other. It’s a new one, it’s better, it’s faster, it’s cheaper.’”

“‘We’re cheaper,’ catches a lot of attention and Ministries of Health will frequently change from doing Rapid Test A in 2005, to Rapid Test B in 2006. And that means everyone has to be re-trained and there are other questions as well, such as ‘have these new kits

been properly validated for the population and the situation that it’s going to be used?’ she said.

### Identifying all the HIV-exposed infants

Another feature of many of the pilot studies is that they were testing infants known to be HIV-exposed. Most of these come from PMTCT programmes, and indeed, one reason for doing DBS is to monitor the effectiveness of PMTCT programmes as soon as possible at their first immunisation visit (and before they are lost to follow-up).

But it goes without saying that uptake of HIV counselling and testing, and subsequent utilisation of PMTCT services varies dramatically from country to country or from site to site within a country. For instance, Botswana can boast that 94% of its HIV-positive pregnant women received either PMTCT or ART in 2006 (Jimbo). But in Tanzania, “over 50% of rural Tanzanian women deliver at home, hampering provision of HIV counselling and follow-up of HIV-positive and exposed infants and the national PMTCT coverage is also low, at 12%,” said Dr Yohanna Abraham of ICAP.

And one key observation made by Dr. Rollin’s surveillance study was that a significant proportion of women with HIV in KZN, South Africa had not accessed PMTCT services at all. In addition, there was another group of women in that survey who reported being tested during pregnancy but who seemed to have seroconverted after that test and who were much more likely to transmit HIV to their infants (possibly because of the high viral loads common during acute infection).

Notably, in a report at the IAS conference last year, use of a serological testing algorithm with a detuned assay for recent HIV seroconversion when applied to samples from the 2005 Sentinel Surveillance exercise in Botswana, detected a startlingly high rate (8% annual incidence) of new HIV infections among pregnant women (meaning, that these women most-likely became infected while they were pregnant) (Moyo). Women are at particularly high risk of infection during and just after pregnancy, and the routine testing offered by PMTCT programmes may not always detect a women’s HIV infection status when she gives birth or is breastfeeding. Programmes must improve prevention counselling and support for those women who test negative.

But the other point is that not only do PMTCT programmes have to improve follow-up and get the HIV-exposed infants that they know about in to be tested, but that many HIV-exposed children aren’t even on the PMTCT programme’s radar screen.

HIV counselling and testing services must be expanded at other entry points in the mother- child health platform: from antenatal services, labour and delivery, immunisation, postpartum care, family planning, and sick and well baby clinic services.

Dr Abraham reported on a pilot programme to increase counselling and testing available in the MCH platform in two district facilities (based upon rapid antibody testing since they did not yet have access to PCR). Over the course of 9 months, the programme succeeded in increasing the numbers of women and children tested (though only 8% of the total visits were tested) and 335 HIV-exposed children were detected and 81% successfully referred into care.

In another ICAP pilot project in Tanzania, DBS for DNA PCR was introduced into 4 sites – and most of the HIV-exposed infants were referred for testing by the PMTCT programme or were referred by an HIV Care and Treatment Clinic. 14% were identified from paediatric wards, MCH, home-based care and VCT sites (Nuwagaba-Biribonwoha).

But in Abraham's study, the results in the sick baby clinic in the study were particularly telling. Over half the number of infants who were delivered eventually wound up in the sick baby clinic (4354). Only 4% of these received testing and counselling, and yet of all the MCH services, the sick baby clinic had the highest percentage of children who tested positive (24% vs 8.5% in the well baby clinic).

In another report, Dr. Sandra !Owoses of the Namibian Ministry of Health and Social Services, stressed that clinical observations alone would lead to an overestimation of transmission among HIV-exposed children, strengthening the case for PCR. Even so, 25.7% of the children with symptoms were infected versus 13% of those without symptoms.

So if programmes are rolling out in early infant diagnosis, and are looking for the kids that the PMTCT programme missed, the very first place to stop must surely be the sick baby clinic.

"But large numbers of mothers are still untested in the MCH and this is because the clinics are congested. There are no additional staff there to offer these services, and with the introduction of new services or a new approach, there are always challenges," said Dr Abraham.

## Remembering to start with the mother first

"We have to test all mothers and infants," said Dr Homsy. "But our national policy and procedure is that no infant should be tested without getting her mother tested first. If the infant comes with a caregiver other than the mother, then it is tested independently of the mother's results. But if the mother is negative, there is no point in testing the infant."

Acceptance of routine HIV testing and counselling among women making MCH visits has been exceptionally high in Tororo, Uganda.

"In terms of consent from the mother, yes, we have less than 2% of the mothers opting out. If it is not documented on the MCH card of the child that [the mother] has been tested and is positive, then we test them and they consent. There is very little opt-out in our setting after routine counselling and testing. Actually when they know they are positive they are extremely concerned to know the result of their child," said Dr. Homsy.

## Community sensitisation and engaging a community response

MCH services can be strengthened to integrate increased HIV counselling and testing by incorporating community-based responses. WHO has put together a resource on this:

[http://www.who.int/child-adolescent-health/publications/CHILD\\_HEALTH/Community\\_IMCI.htm](http://www.who.int/child-adolescent-health/publications/CHILD_HEALTH/Community_IMCI.htm).

Community-based mechanisms are important in getting mothers and, if necessary, their infant tested so that both get linked to care, according to Dr Addy Kekitiinwa of Baylor College of Medicine Children's Foundation-Mulago Hospital in Uganda, who described a project trying to better link PMTCT with MCH services.

"We had peer HIV-positive mothers identify newly diagnosed HIV-positive women at antenatal clinics and then introduce HIV infected-pregnant women to the MCH clinic. The peer mothers were vital in implementing a successful PMTCT linkage."

"In Botswana, we also have a cadre of lay counsellors who go into the community to mobilise the mothers to bring the infants so that they can be tested in good time," said Dr William Jimbo of BotUSA

"You have to make a link with your community," said Dr Gonzalez. "You have to take your community based organisation and train

them how to go to take a look — they know which mother is pregnant and where they delivered. And try to listen."

"We have a programme officer and a nurse coordinator for the programme based within the Great Lakes region," said Dr Harriet Nuwagaba-Biribonwoha of ICAP in Tanzania. "They are now well known within the community and they've actually been to churches, to community meetings, to mosques and trying to tell people about these programmes and trying to involve them. And that involvement has included both mothers and fathers," she said.

## Unique infant identifiers

Once an HIV-exposed infant has been identified, another key challenge is keeping track of it and matching any testing samples to the patient.

In Ethiopia, "during the launch of the DBS programme, regional NGOs and partners working at different levels developed an infant unique identifier system for identification of exposed infants, testing them and linking them into treatment programmes," said Dr Hailegiorgis who also works with ICAP.

Upon first identifying an HIV-exposed infant, each child is given a unique infant diagnosis identifier that is put onto the child's health card (tracking him or her through the various programmes) and which is used to identify the child on all samples sent for testing (including repeat samples). This number is used to track the samples, and the system can include additional information relevant to the child or the programme (much more detailed information on setting up such a system and logbooks is available through ICAP's Manual on Infant Diagnosis (see <http://www.columbia-icap.org/resources/peds/files/Infantdx050307.pdf>).

## Training

To scale up implementation of DBS, more training will be needed.

"We need to have core trainers," said Dr Mudany, "by doing training of trainers and picking out master trainers who can go around training within the regions."

Dr Homsy pointed out that the training should engage the entire range of healthcare workers, from the laboratory personnel to the midwives.

"In our case, it's the midwives who do the rapid tests and DBS collections, and after the quality assurance that we carried out, we found that the midwives did systematically better than the lab techs!"

Much of the training across the PEPFAR focus countries starts over the border.

After ICAP's experience with PCR in Rwanda, Dr Gonzalez said "we bring in the technicians from other countries to Rwanda to be trained in one month/one month and a half with senior technicians and it is working very well," he said.

## A dedicated space for DBS collection

Since DBS is only being collected on HIV-exposed babies, there is a chance that it could lead to HIV disclosure, if it is not done in a private room.

"Initially, there was no dedicated space for exposed-infant diagnosis which caused a loss of confidentiality in some cases so these are now developed immediately," said Dr. Hailegiorgis.



## Getting the DBS samples to the centralised lab – establishing a routine, reliable and rapid transport service

A key feature of the DBS system is that the samples can be sent to the lab in an envelope, without refrigeration. However, there is little point in collecting the DBS specimens, sticking them in a drawer or shelf for weeks or even months and then sending them to the lab (a common problem if staff are not adequately trained or provided with a routine way to send samples to the lab).

"We need to put in place an effective transport system that links up the facilities with a laboratory," said Dr. Mudany.

This is more challenging in some settings than others and Dr Gonzalez suggested that each programme must work out the logistics for transport that works best in its setting (especially for more remote sites).

"In the case of Rwanda, we use the same car to move from Kibuye to the capital taking the medicines, the reagents for the CD4 – or whatever – once a week. You just put everything together in the same car, and we give the envelope with the DBS to the driver and the driver will drop the samples into the national laboratory and the same transportation brings the results back. Or you have a DHL system like in Botswana and that's a good way to do it there. But for Rwanda, our system works very well... and the cost of transportation is zero, because you use the transportation that already exists."

But he added that Rwanda is a small country with the capital (and the lab) right in the middle of it. And the transportation infrastructure in many countries is particularly poor, especially during the rainy season.

In a place like Ethiopia, it's a different question altogether. The first phase of the DBS roll-out in that country utilised one centralised lab in Addis Ababa, and some samples were routinely transported from as far as 770 km away to the central lab – even though regional labs are now being brought online. "Specimen transport is still a challenge because of the long distances," said Dr Hailegiorgis. "Fast, affordable, reliable and sustainable courier systems for specimen transport are required when PCR testing is centralised."

## Long turn-around times and the need for well-coordinated laboratory strengthening and quality control

Another challenge that many programmes scaling up DBS seem to be facing is that laboratories cannot immediately meet the new demand and there were often backlogs at the lab. Dr Homsy reported a PCR turn-around time of 70 days during his study, though he said that was due to an initial backlog that should now be resolved.

There is a danger that ability to collect and deliver DBS to reference laboratories could outstrip the capacity in the labs, if those are not strengthened, supply chains established, and/or new facilities brought online fast enough.

For instance, in trying to scale-up implementation nationwide, Botswana's programme collected over 6,500 DBS between November 2006 and May 2007 – but had only tested and delivered results to the districts for 3,135 samples, due to delay in opening a second lab, and reagent supply interruptions.

Mr Madisa Mine from the Botswana HIV Reference Lab also stressed that a successful pilot project does not necessarily lead to

successful roll-out without training more technicians and without adequate resources being allocated to improving laboratory capacity.

And he pointed out that the laboratory must continue to do its regular work while taking on these new tasks, and that quality control and assurance must be ongoing.

This again raises the issue of how limited laboratory capacity should be allocated in countries.

In South Africa, which is much better resourced than most countries, lab capacity has grown by leaps and bounds. Even so, the demand is so great, and access can still be difficult for rural clinics that often serve thousands of people with HIV. So one of the responses has been to bring PCR capabilities closer to the bigger remote clinics.

"One solution that we have tried is a laboratory in an adapted ship's container," said Dr Linda Gail Bekker of the Desmond Tutu HIV Centre at the IAS meeting. "It has its own generator [particularly important in South Africa now that the power supply has become unreliable] and we estimate it would be able to serve 10,000 patients. We are testing it in a peri-urban situation and we do all the viral load and toxicity measurements and infant diagnosis testing there for a 4000 patient clinic in this container. And we have been able to show excellent quality assurance and quality control in this lab, compared with central laboratories."

"Our greatest challenge is more rapid access to the results once the specimens have been taken. There is a considerable time delay (partly because of distance and partly because of workload). Ideally we need a point of care test for children," said Dr Halima Darwood of Grey's Hospital in KwaZulu-Natal.

## Delivery of results and follow-up

A couple of programmes reported that parents don't always come back for the test results even when the turn-around is relatively short.

"We have a challenge with mothers/parents who are not coming back for results," said Dr Nuwagaba-Biribonwoha, "maybe because the infants are negative, maybe because they are thinking the infants are not ill but they don't come back."

Again, community linkages and community-based follow-up are vital. Many South African sites are working with groups such as mothers2mothers, a peer-based programme that provides education and support for pregnant women and new mothers, which may provide the most efficient way to retain these mothers and infants in care (see <http://www.m2m.org/>).

## Post-test counselling

Counselling also has to be carefully adapted to address the concerns of parents whether the test result is positive and negative.

"We have heard of reported parental neglect of some of the HIV-positive infants," said Dr Karanja. He also reported that women whose babies turn out HIV-negative often believe that means that if they get pregnant again, their next babies will also be HIV-negative – which isn't always the case. "There is a high desire for conception of HIV-negative babies. So we need to improve on our counselling skills so that they make informed decisions."

## What about breastfeeding?

But the biggest counselling issue concerns the dangers that women may stop breastfeeding their infant if they are told the result is negative, long before the woman has found replacement feeding



options that are acceptable, feasible, affordable, sustainable and safe (AFASS).

"One of the real issues that we have by testing the infants at 6 weeks is that we risk early weaning. And we have data that clearly shows that HIV-negative babies who are weaned early die prematurely," said Dr Homsy (see also <http://www.aidsmap.com/en/news/1C6972FE-B40B-42BC-BC71-2BA36589535E.asp>).

"If we test the baby and the baby is negative, we need to accompany that with very good counselling on infant feeding. We know from the recent guidelines by WHO that exclusive breastfeeding is protective and is important for nutrition where a mother does not fit the AFASS criteria. So especially in most of Africa, where most of our mothers cannot afford safer alternatives – we should still talk about exclusive breastfeeding. There is not as much transmission if they can do exclusive breastfeeding rather than mixed feeding. So the test results should be accompanied by good counseling on nutrition," said Dr Mudany.

But Dr Nuwagaba-Biribonwoha of ICAP in Tanzania said that the problem of premature weaning may begin even before testing the infant.

"We had at least 100 children, who by the time we do the first DNA PCR, were not breastfeeding. So I think – not only at the time we receive the results, but also during PMTCT – that the messages about exclusive breastfeeding have to really be reinforced," she said.

Part of the problem is that there are a lot of mixed messages being put out about breastfeeding, with some projects going to great lengths to introduce formula feeding in some resource-limited settings.

In another presentation at the Implementers' Meeting, Dr Saul Onyanga, from Kampala Uganda, said that programmes needed to send less ambiguous messages about infant feeding. "If you look at a small programme at health facility level, one small project, it is very possible to intervene with formula feeding and support that community. But not at the national programme level."

"I agree with what people have said in terms of reinforcing the counselling strategy but this also reinforces the need to put mothers who are eligible on ART," said Dr Homsy. "We know that these mothers are the most likely to transmit because of the high viraemia. I don't think we have any arguments with breastfeeding at this point – the point is to make breastfeeding safe. And if we don't make that effort - putting the mother on treatment before they deliver, then we raise the chance of transmission."

Very reassuring data from two studies presented at the IAS meeting demonstrated that ART dramatically reduces the chances that breastfeeding women with HIV will transmit the virus to their infants (to below 1%, see <http://www.aidsmap.com/en/news/4920CF7D-9E95-4C08-85A1-65F001CB6E60.asp>) (Kilewo, Arendt).

## Effective transition to care

The real point of these testing protocols should be to get children into treatment and care.

"We can do all the diagnostics that you want but if we do not put babies on treatment, why are we doing diagnostics?" said Dr. Gonzalez. "This is for medical intervention – we have to put those babies on treatment because that's the main reason to do early infant diagnostics."

"After identification, we have to actively make certain these HIV-exposed infants get into care and treatment, so [early infant

diagnosis] must be closely integrated with clinical training, mentoring and supervision," said Dr Hailegiorgis. He noted that when his programme started: "There was lack of coordination of lab and clinic activities at the beginning because those results which came back from the laboratories were not analysed in the beginning."

Dr Gonzalez recommended training a focal person at each site who would be responsible for following early identification of HIV-exposed infants, tracking them through diagnosis, and making certain that their results are delivered and that the children are entered into care and treatment.

But in many locales, as Dr. Karanja noted "there is a lack of adequate facilities that offer care, support, and treatment for HIV-infected infants."

In Botswana, where the ART programme is particularly aggressive, about 35% of the infants who tested positive for HIV have been put on ART, and another 30% still in follow-up according to Dr Jimbo. But he reported disheartening outcomes for the rest, who either died before starting ART (7%), or their families refused to put them on ART (8%), or the infant was referred for ART but the outcome is listed as unknown (10%) or the infant simply could not be found.

Dr Angela Mushavi of Katatura State Hospital in Namibia said delays in getting HIV-positive children onto ART could lead to poor outcomes.

"My experience with the HIV-DNA is the unfortunate incidents where we have a positive HIV DNA-PCR at 6 weeks, you go on to do clinical staging and CD4 criteria and the child is not yet eligible for ART. And then weeks later the child comes in and is dead! What are we doing for those children? Maybe we should be making a case for treating all HIV-positive infants instead of waiting until we actually have children dying on our hands," she said.

## MCH systems strengthening

Again, to get the most benefit out of early infant diagnosis, investment must be strengthened in the entire MCH platform that provides care to mothers and infants in resource-limited settings.

Trying to implement or strengthen just one aspect of a programme, implementing DBS on its own, for instance, comes with risks, as was shown by a recent paper in JAIDS, which reported that when routine PMTCT service programmes were integrated into clinics in Zambia, delivery of other routine clinical services for pregnant women (as gauged by syphilis screening) suffered (<http://www.aidsmap.com/en/news/9C6E664F-5029-4968-BFD7-A3BC3A083FB6.asp>). Dr Hailegiorgis made a similar observation during the implementation of early infant diagnosis in Ethiopia.

"We had some challenges during this process with human resource issues because new tasks had to be given to PMTCT and primary care nurses and this has compromised their workflow, and there was also scaling up of specimen collecting which was at the expense of other activities," he said.

In order to avoid the danger of diverting existing resources towards DBS, we have to extend the capacity to deliver better maternal-child health on multiple fronts in tandem.

## Looking forward

Some participants at last year's major conferences were looking towards a future where infant diagnosis will be easier and can be delivered on the spot.

"I commend the efforts but a better test is needed that could be done at the site of the infant with a result right so we need to work

on it," Dr Renee Ridzon from the Gates Foundation said at the HIV Implementer's meeting.

Any progress that the Gates Foundation and/or commercial enterprises can make towards developing an inexpensive point-of-care HIV test that works in infants is more than welcome. But in her report on the state of the art in the field at the IAS conference one month later, Prof. Susan Fiscus wasn't too encouraging.

"There's an awful lot of activity in this area but nothing that would be ready to be used today. This is the future and I do think this is where we are going but I don't think we're there yet," said Prof Fiscus.

So in the meantime, specimens will still have to be transported to centralised labs to be tested. Perhaps further study will confirm the clinical utility of using standard viral load tests, which, if successful would streamline the process of laboratory scale-up.

However, it is possible that a less expensive alternative to PCR testing could eventually be performed on the samples. Two are worth noting: the Cavid Reverse Transcriptase (RT) Assay (ExaVir Load version 3), and the heat dissociated p24 antigen (hdP24A) assay.

ExaVir Load uses standard ELISA laboratory equipment plus a reader from Cavid to look for the HIV enzyme reverse transcriptase (and it should work with any subtype). Data presented at the IAS meeting suggest that the current version of the test is more sensitive and can now detect viral loads over 2000 copies/ml (Greengrass).

Finally, there has been recent progress on using the hdP24A assay on DBS. This test would be much cheaper because "the equipment is generally available, it's less technologically complex; it's less prone to contamination compared to PCR; it seems to be excellent for infant diagnosis; it's very reproducible and it works quite well with DBS. But it's still not as sensitive as the DNA assay if the mother of the infant is on ART, and we need more information on some of the subtypes," said Prof. Fiscus.

Another problem, pointed out by Dr Homsy at the Implementers' meeting is that "the test is not a commercially available assay, and furthermore, it's difficult to roll out because every lab has to have its in-house standards made before you can use it," he said.

But perhaps a way could be found by some public-private partnership to package a standardised kit for hdP24A in much the same way that the Global Drug Facility is packaging "smear microscopy kits" for TB (see <http://www.aidsmap.com/en/news/E398B0C4-01D1-4946-B219-39741A8618A8.asp>). The Foundation for Innovative Diagnostics at one time discussed doing something similar to package a safer version of MODS liquid culturing system for TB.

But while work on newer and hopefully inexpensive technologies progresses, Dr Souleymane Sawadogo from CDC in Namibia who moderated the HIV Implementer's Meeting succinctly summed up the need to scale-up existing laboratory capacity and roll-out DBS now. "The thing is that babies are dying. They can't wait for us... we have to push everything at the same time."

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