Early detection of HIV infection in infants and children

Guidance note on consideration of options for selection of technology for early diagnosis of HIV in infants in resource-limited settings.

Summary Recommendations

- WHO recommends that national programmes establish capacity to provide early infant virological testing for HIV given the high risk of death by 2 years of age for infected infants and the increasing availability of paediatric antiretroviral treatment in many resource limited settings.

- Currently available assays and diagnostic platforms that should be considered by MOH programs for early infant HIV diagnosis include appropriately ongoing externally validated commercially and non-commercially available tests for HIV DNA (PCR) or HIV RNA.

- Laboratory capacity to perform virological testing using HIV-DNA (PCR) and/or HIV-RNA should be made available at least at central or tertiary level and operationalized to facilitate national coverage by additional laboratory testing capacity or through referral.

- Serological assays suitable for HIV antibody detection in adults cannot be reliably used for confirmatory diagnosis of HIV infants under 18 months as interpretation of positive HIV antibody testing is complicated by the fact that maternal HIV antibody can persist as long as 18 months (although usually maternal antibody clears by 9-12 months of age).

<u>Context</u>

- Early virological diagnosis of HIV infection in infants and children enables :
    - ✓ early identification of infants and children who are HIV-infected as a first step in securing their treatment and care;
    - ✓ identification of HIV-exposed infants and children who are uninfected facilitating follow-up care and prevention measures that will help to ensure they remain uninfected;

- ✓ assists in the effective use of essential resources by targeting ART to those who need treatment ;
- ✓ improves psychosocial wellbeing of family and child, including reducing potential stigma, discrimination and psychological distress for those HIV-uninfected children and increasing the chances of adoption for orphans;
- ✓ facilitates life-planning for parents and/or children who are HIV-infected.

Available Technology

- HIV-DNA Polymerase chain reaction (PCR) is the most widely used assay in industrialised countries for early infant diagnosis.
- HIV RNA (PCR and other nucleic acid detection techniques) has also been shown to be accurate and reliable, and provides additional information about virological status.
- Both HIV-DNA and HIV RNA technologies are becoming less expensive, more automated and faster in producing results.
- Ultrasensitive p24 antigen detection is a reliable method for detection of HIV infection in infants over 6 weeks of age but the required equipment and consumables are currently not available for purchase in ways suited for use in achieving national programme coverage.
- As resource-limited settings are increasingly able to purchase HIV DNA/ HIV RNA kits and equipment, their choice of technology should be guided by potential public health benefits, and needs to consider the following additional factors:
    - ✓ new purchase of required equipment
    - ✓ commercial availability and cost of equipment and reagents
    - ✓ projected numbers of tests required from specific geographical areas
    - ✓ projected number of samples required to be processed (sample throughput)
    - ✓ specimen storage and transport
    - ✓ ongoing laboratory quality assurance
    - ✓ availability of maintenance and service for equipment and supplies
    - ✓ sample collection and processing (including DBS specimen to machine)
    - ✓ other equipment required (e.g. automated specimen preparation, DBS punchers, centrifuge)
    - ✓ viral types and subtypes
    - ✓ training and availability of laboratory staff
    - ✓ use of the equipment for other purposes (e.g. diagnosis of other conditions or monitoring of ART)

WHO can provide technical guidance on the above issues, and may assist in purchase of technology at reduced prices through its Bulk Procurement Schemes (see contacts at the end of the document).

Context: Paediatric HIV/AIDS in resource-limited settings

For the foreseeable future, at least a half million HIV-infected infants will be born each year, most of them in low-income countries with generalized epidemics. Mother-to-child transmission of HIV accounts for the vast majority of the world's estimated 2.3 million children under the age of 15 years living with HIV, (1.7–3.5 million), almost 90% of them in sub- Saharan Africa. 780 000 (600 000–1 000 000) were estimated to be in need of antiretroviral therapy, and in 2006 it was estimated that 380 000 children under 15 years of age (290 000–500 000) died of AIDS-related causes.[1, 2]. Despite a 40% increase in the number of children receiving ART in 2006 children , children comprise only 6% of people on treatment globally whereas the percentage of people in need of treatment who are children is 14% [2]. National programmes that are able to report by age reveal that very few of these are children under 2 years of age.

While affordable ART and treatment for opportunistic infections are becoming increasingly available, this is of little benefit to infants unless they can be diagnosed early. Most infants who are HIV infected will die before reaching their second birthday, and about 33% will die before reaching one year of age[3-5]. Unfortunately, interpreting results from the most widely available serological (antibody) assays used for adults are difficult in infants under 9-12 months of age. Where HIV antibody negative they do suggest the child is uninfected, but due to the persistence of maternal antibody in children born to HIV infected mothers, in rare cases for up to 18 months, virological testing is the required method of diagnosis of HIV infection in infants and children aged under 18 months.

Why early diagnosis is crucial

Early diagnosis of HIV infection allows health care providers to offer optimal care and treatment of the HIV-infected child, assists in decisions on infant feeding, and avoids needless stress on mothers and families. With increasing efficacy and coverage of PMTCT interventions, the majority of children born to HIV infected mothers will be uninfected (with effective ARV/ART interventions > 90%), so recognizing those with infection before they become unwell is only possible through routine diagnostic testing, ideally at PMTCT or Maternal and Child Health services. WHO recommend this be performed with virological testing at any age from 6 weeks of life.

With no interventions about 5 - 20% of infants of HIV-positive mothers become infected through breastfeeding. There is evidence that exclusive breastfeeding in the first months of life is safer than mixed feeding. Where the mother is on ART for her own health, transmission through pregnancy and breastfeeding is likely to be reduced. Early diagnosis also assists in decisions about breastfeeding. An

HIV-positive mother with an HIV uninfected baby can be counseled and supported to stop breast-feeding if replacement feeding is acceptable, feasible, affordable, sustainable and safe (AFASS)[i]. If the baby is HIV infected, the mother can be counselled and supported to continue breast- feeding. Finally, early diagnosis of HIV in infants assists the family in life planning.

Waiting for children to become unwell to use virological testing, while accurate, delays diagnosis of HIV. Clinical algorithms are not reliable, and have poor predictive value in young children especially in the first year of life. Clinical algorithms plus CD4 testing in experienced hands appear to provide better diagnostic discrimination, but further research is needed.

Existing programmatic gaps and obstacles

As programmes plan for programme use of virological diagnostic equipment a number of factors need to be considered:

Laboratory-related issues

- limited number of laboratories with capacity to perform virological testing;
- relatively high cost of commercially available tests and associated equipment (see sources and prices[ii]);
- difficulties in ensuring required laboratory conditions e.g. secure power supply, provision of cold storage, supply chain, maintenance service and spare parts;
- weak systems for specimen distribution and reporting of results;
- lack of the laboratory quality control systems required to ensure accuracy of results;
- lack of international regulatory approval for existing diagnostic platforms;
- lack of commercially available reagents and consumables for some diagnostic platforms;
- lack of access to appropriate training for technical staff;

Programme-related issues

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[i] See WHO/UNICEF 'HIV and infant feeding: Framework for priority action (English)': UNICEF and WHO recommend that "when replacement feeding is acceptable, feasible, affordable, sustainable and safe, avoidance of all breastfeeding by HIV-infected mothers is recommended. Otherwise, exclusive breastfeeding is recommended during the first months of life" and should then be discontinued as soon as it is feasible.

Weblink: http://www.who.int/child-adolescent-health/New_Publications/NUTRITION/HIV_IF_Framework_pp.pdf:

[ii] http://mednet2.who.int/sourcesprices/sources.pdf

- poor utilisation of ANC and child health services;
- lack of knowledge of maternal HIV status;
- poor coverage of PMTCT services;
- poor post-natal follow of HIV-exposed babies;
- lack of human resources to ensure linkages and referral between PMTCT ANC and child health services;
- Lack of recognition or consideration of HIV in children.

Polymerase Chain reaction (PCR) and other Nucleic acid detection technologies

Among the technologies available for diagnosis of HIV in infants, polymerase chain reaction (PCR) on DNA in blood is currently the most widely used, and is generally considered the standard method. Recent studies have also demonstrated evidence that real time PCR for HIV RNA provides a reliable and suitable alternative.

HIV-DNA PCR is a qualitative test (i.e. this gives a yes/no diagnosis of HIV infection). HIV RNA detection provides additional quantitative information on the infant's virological status, and the same technology/equipment is used for monitoring response to treatment and possible therapeutic failure. Commercial assays for the detection of HIV RNA are now more widely available than DNA assays. None of the testing platforms (i.e. DNA or RNA) have licensing approval for use in routine diagnostic services with US or European regulators, although this may change over the next few years.

Both DNA and RNA technologies are complex and expensive methods requiring dedicated equipment, space and trained technicians. However, increased automation remarkably reduces the technical challenge. Some DNA and RNA (PCR) technologies support the use of dried blood spot (DBS) samples, which have considerable advantages in settings were sample taking and transportation and storage is problematic.

Ultrasensitive p 24 antigen Assay

Diagnosis of HIV infection can also be made by measurement of immune complex dissociated p24 antigen, as this marker is specific for HIV infection. This is an ELISA based technology, and therefore less expensive and in principle requires less stringent laboratory capacity. However, in practice the performance of the currently available tests does require a skilled lab technician. In addition, the equipment and most reliable required consumables are not commercially available together. With the considerable drop of prices for PCR the price advantage of the ultrasensitive p 24 antigen tests has become smaller.

Timing of early virological testing

Regardless of the type of virological testing technology used the following should be considered:

- Virological testing at 6 weeks of age gives a good sensitivity (>98 %) with the various methods and is considered programmatically more efficient.
- Good practice would be to confirm a positive PCR result on a second specimen - but for clinical management purposes a confirmed positive test result from one specimen or a repeat test on the same specimen is viewed by experts as sufficient provided that laboratory quality assurance is guaranteed.
- Performing one early HIV virological detection test at six weeks for all HIV-exposed children will identify the bulk of the children infected prior to, during and immediately after delivery, and therefore identify the majority of the babies who will progress rapidly and go on to need life saving ART.
- Testing before the age of 6 weeks using the DNA and RNA detection methods can detect HIV in infants infected in utero, however is not recommended for routine national programme use.
- Timing of any repeat testing should also consider breast feeding practices as the risk of acquiring HIV infection from the mother continues through the entire breastfeeding period.
- Virological testing is also required for children presenting to health care services with signs and symptoms of HIV infection, as this is where the bulk of HIV cases will be present given current low PMTCT coverage. From 9 months of age testing with HIV antibody tests first is recommended to ensure virological testing is only performed on those children who still have HIV antibody.

Factors to consider in selecting national diagnostic technology for early detection of HIV in infants and children

A range of factors should be taken into account in considering the purchase of diagnostic kits and equipment, and in choosing or upgrading a virological laboratory to operate the diagnostic service.

Location of testing facilities

Deciding where and how many testing facilities are optimum is partly a question of national burden of HIV and required coverage and capacity. Procuring RNA or DNA (PCR or other) testing equipment for a small number of laboratories offers potential benefits such as greater cost-effectiveness, greater possibilities for automation, the development of specialist expertise, robust infrastructure (e.g., continuous power supply), timely access to equipment maintenance, and secure consumables supply.

On the other hand, centralization of virological testing capacity may make testing specimens from remote regions slow and reduce the quality and reliability of service. The optimum configuration will depend upon factors outlined below, and will be different in each country.

Workload and sample throughput

Predicted workload and the frequency and number of specimens to be tested in a given period are crucial to assessing cost-effectiveness and suitability of any given technology. Some assays are better suited to larger throughput, and may lead to wastage if not fully utilized. Consideration needs to be given to the immediate situation and the estimation of needs over the longer term (2-5 years). Unless the test is performed regularly it will be difficult to maintain the required level of competency amongst technicians, and more equipment-associated problems are likely to occur.

Increasingly PCR methods are becoming automated, thus reducing potential for errors and increasing the reliability of results. In addition real time PCR is shortening considerably the procedure time, allowing for greater throughput and more rapid reporting of results. Depending on the predicted workload, manual or automated sample preparation may be considered. Many laboratories start off with manual sample preparation and invest in full automation if workload increases. Although more expensive than manual extraction methods, fully automated diagnosis is less technically demanding and thus offers greater reliability and greater throughput per technician.

Sample collection and transportation

Viral nucleic acids may degrade over time, and so the type of specimen and time between specimen collection and testing is important, particularly if stored at high ambient temperature (e.g. during transportation to the laboratory) for extended periods. Specimens can be protected if transported rapidly at a cool temperature (2-10^{o}C). However, where transport and refrigeration are problematic such as in remote or rural areas, dried blood or plasma spots (DBS) may be the best solution.

It is essential that the procedures be validated after implementation. For example, if DBS are collected and transported to the diagnostic laboratory by mail, the promptness and reliability of the model should be evaluated at each point in the chain of events (collection, mailing in, testing, reporting back, etc.).

Human resources

High quality diagnostic services require staff with appropriate education and training. Currently, few countries with high-HIV prevalence have sufficient numbers of technicians, administrators, maintenance workers and others to fully support a scaled up diagnostics network. For this reason, provision should be made for training (and retaining) such valuable human resources through a

detailed plan and budget based on realistic objectives. Where staff capacity is low, a higher degree of automation may be able to compensate for this, but only if maintenance and repair of the equipment can be assured.

Laboratory facilities and infrastructure

When selecting or equipping a laboratory to undertake HIV virological testing, a variety of factors must be taken into consideration. Physically, the laboratory building should have reliable water and power supply, and appropriate storage facilities including refrigeration and freezer capacity.

Good laboratory procedures must be in place, guided by a code of practice and well-documented standard operating procedures and a quality management system. Responsibilities for each staff member should be defined and documented within a clear management structure. The staff should include a quality manager, a training officer and a health and safety officer, although these roles may be carried out by existing staff as additional responsibilities. Appropriate health and safety regulations should be backed up by effective training programmes and procedures for activities such as decontamination, sharps disposal, emergency procedures, and waste handling (incineration is the preferred method for disposal of PCR products). Maintenance of equipment and supplies must be supported by equipment maintenance logs, rigorous stock control procedures, safe and accurate means of labelling and transporting specimens, and good record keeping and reporting.

In order to keep quality of service high, the facility should participate in both external and internal quality assessment schemes, and institute ongoing evaluation of laboratory performance. WHO and the US Center for Disease Control (CDC) can provide technical support.

Procurement, supply and maintenance

A number of factors regarding procurement of commercially available assays must be taken into account. For most resource-constrained settings, the technology chosen should support the use of dried blood spot samples, supported by standardized and validated protocols. Some commercially available PCR platforms permit measurement of Hepatitis C and Hepatitis B, other sexually transmitted infections, and *M. tuberculosis*. Such "multiple use" may make the purchase of a given assay even more cost-effective.

It is often possible to negotiate a favorable price through bulk procurement, and it may therefore be advisable for price negotiations to be carried out at a national level. The WHO Bulk Procurement Scheme may be helpful in this regard. The suppliers must be able to guarantee the timely provision of test kits and associated reagents and consumables, with an adequate shelf life. The negotiated package should include technical support by the supplier, initial training in the use of the reagents and equipment (including routine equipment maintenance tasks to be completed by the testing facility's

staff), and regular scheduled maintenance and emergency repair by specialists of any associated equipment supplied.

Because of the cost and complexity of the technologies, laboratory managers should take a long-term view in their procurement and supplies management, including forecasting needs, staggered deliveries ensuring sufficient stocks without wastage, and proper storage. Close monitoring of the stock will allow flexibility to accommodate increased demand and avoid interruption of supplies which would otherwise result in interrupted service.

For further information

As prices and assay features are changing rapidly, it is advisable for decision-makers and specialists in this area to keep to date with current information. The website of WHO's Diagnostics and Laboratory Technology (http://www.who.int/diagnostics_laboratory/en/) is a useful resource, with information about evaluated assays, bulk procurement, quality assurance, and guidance and training.

The HIV departments' web site has a web page on paediatric HIV infection (http://www.who.int/hiv/paediatric/en/index.html).

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