Early detection of HIV infection in infants and children

Guidance note on the selection of technology for the early diagnosis of HIV in infants and children

Summary of recommendations

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

- Assays and diagnostic platforms that should be considered by health ministry programmes for early infant HIV diagnosis include externally validated tests for HIV DNA or HIV RNA (including polymerase chain reaction, PCR) which are commercially and non-commercially available.

- Laboratory capacity to perform virological testing using HIV DNA PCR and/or HIV RNA (PCR or other methods) should be made available, at least at the central or tertiary level, and should be 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 in infants as the interpretation of positive HIV antibody testing is complicated by the fact that maternal HIV antibody can persist for 18 months (although it usually clears by 9−12 months).

- Antibody-negative results suggest that infants are unexposed and or uninfected, however if the infant is breastfeeding the risk of acquiring HIV continues throughout the entire breastfeeding period.

Context

- Early virological diagnosis of HIV infection in infants and children:
    - ✓ enables the early identification of who have HIV-infection, as a first step in securing their treatment and care;
    - ✓ enables the identification of those who are HIV-exposed but uninfected, facilitating follow-up care and prevention measures that will help to ensure that they remain uninfected;
    - ✓ assists in the effective use of essential resources by targeting ART on children who need treatment;
    - ✓ improves the psychosocial well-being of families and children, reducing potential stigma, discrimination and psychological distress for HIV-uninfected children and increasing the chances of adoption for orphans;
    - ✓ facilitates life-planning for parents and/or children who have HIV.

Available technology

- HIV DNA PCR is the most widely used initial assay for early infant diagnosis in industrialized countries.
- HIV RNA PCR and other nucleic acid detection techniques have also proved accurate and reliable, and they provide additional information about virological status that can assist in clinical management.
- 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 aged over 6 weeks but the required equipment and consumables are not yet available for purchase in ways suitable for use in achieving national programme coverage.
- As resource-limited settings are increasingly able to purchase HIV DNA and HIV RNA kits and equipment, their choice of technology should be guided by potential public health benefits. The following additional factors should also be considered:
    - ✓ 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 transfer of dried blood spot (DBS) specimens to machines;
- ✓ other equipment required, e.g. automated specimen preparation, DBS punchers, centrifuges;
- ✓ viral types and subtypes;
- ✓ training and availability of laboratory staff;
- ✓ use of 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 the purchase of technology at reduced prices through its Bulk Procurement Schemes (see http://www.who.int/diagnostics_laboratory/procurement/viral_load/en/index.html and contacts at the end of this document).

Paediatric HIV/AIDS in resource-limited settings

It is to be expected that, for the foreseeable future, at least 500 000 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 2.3 million children under the age of 15 years who are estimated to be living with HIV (1.7–3.5 million) , almost 90% of them in sub-Saharan Africa. It has been estimated that, of these children, 780 000 need antiretroviral therapy (ART), and that, in 2006, 380 000 children aged under 15 years died of AIDS-related causes [1, 2]. Despite a 40% increase in the number of children receiving ART in 2006, children comprise only 6% of the people on treatment globally whereas 14% of the people in need of treatment are children [2]. National programmes that are able to report by age reveal that very few of these children are under 2 years of age.

Affordable ART and treatment for opportunistic infections are becoming increasingly available but this is of little benefit to infants unless they can be diagnosed early. Most infants who are HIV-infected die under the age of 2 years and about 33% die under the age of 1 year [3−5]. Unfortunately, interpreting results from the most widely available serological (antibody) assays used for adults is difficult in infants aged under 9−12 months. Antibody-negative results suggest that infants are uninfected. HIV antibody positive results do not confirm infant infection because maternal antibody in children born to HIV-infected mothers may persist; virological testing is therefore the required method of diagnosis of HIV infection in infancy. Breastfeeding, while associated with improved survival

puts the infant at risk of acquiring HIV throughout the entire breastfeeding period, even if infant is initially uninfected (see http://www.who.int/child-adolescent-health/New_Publications/NUTRITION/consensus_statement.pdf).

Why early diagnosis is crucial

Early diagnosis of HIV allows health-care providers to offer optimal care and treatment of HIV-infected children, assists in decision-making on infant feeding, and avoids needless stress in mothers and families. The increasing efficacy and coverage of PMTCT interventions mean that the majority of children born to HIV-infected mothers will be uninfected (with effective ARV/ART interventions exceeding 90%). Consequently, recognizing those with infection before they become unwell is only possible through routine diagnostic testing, ideally in services for PMTCT or maternal and child health. WHO recommends that this be performed with virological testing at the age of 6 weeks or any time subsequently, initiated by the responsible health care providers as outlined in WHO guidance on provider initiated HIV testing (for WHO Guidance on Provider-Initiated HIV Testing And Counseling In Health Facilities see http://www.who.int/hiv/en/).

If there are 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. If mothers are on ART for their own health, transmission through pregnancy and breastfeeding is likely to be reduced. Early diagnosis also assists in decision-making on breastfeeding. An HIV-positive mother with an HIV-uninfected baby can be counselled and supported to stop breastfeeding 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 breastfeeding. Finally, early diagnosis of HIV in infants assists families in life-planning.

If virological testing is only performed after children have become unwell the results are accurate but the diagnosis of HIV is delayed. Clinical algorithms are not reliable, and have poor predictive value in young children, especially during the first year of life. Clinical algorithms plus CD4 testing in experienced hands appear to provide better diagnostic discrimination but further research is needed.

[i] See: WHO/UNICEF. *HIV and infant feeding: Framework for priority action.* UNICEF and WHO recommend that, when replacement feeding is acceptable, feasible, affordable, sustainable and safe, all breastfeeding by HIV-infected mothers should be avoided. Otherwise, exclusive breastfeeding is recommended during the first months of life and should then be discontinued as soon as feasible. http://www.who.int/child-adolescent-health/New_Publications/NUTRITION/HIV_IF_Framework_pp.pdf:

Programmatic gaps and obstacles

As programmes plan to use virological diagnostic equipment the following 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, spare parts;
- weak systems for specimen distribution and reporting of results;
- lack of 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:

- poor utilization of ANC and child health services;
- lack of knowledge of maternal HIV status;
- poor coverage of PMTCT services;
- poor postnatal follow-up 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 and other nucleic acid detection technologies

Among the technologies available for diagnosis of HIV in infants, PCR on DNA in blood is the most widely used and is generally considered to be 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. it gives a yes/no diagnosis for HIV infection). HIV RNA detection provides additional quantitative information on virological status, and the same

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

technology/equipment is used for monitoring the 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 United States or European regulators, although this may change over the next few years.

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

Ultrasensitive p24 antigen assay

The diagnosis of HIV infection can also be achieved by measuring immune-complex-dissociated p24 antigen, as this marker is specific for HIV infection. This is an ELISA-based technology, and is therefore less expensive than other nucleic acid technologies and, in principle, requires less stringent laboratory capacity. However, in practice the performance of the available tests demands skilled laboratory technicians. In addition the equipment and most of the required and reliable consumables are not commercially available together. With the considerable fall in costs for PCR the price advantage of the ultrasensitive p24 antigen tests has become smaller.

Timing of early virological testing

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

- One early HIV virological detection test at or after 6 weeks of age for all HIV-exposed children identifies most children infected before, during and immediately after delivery, and therefore identifies most babies who will progress rapidly and who will need life-saving ART.
- Virological testing at 6 weeks of age gives a good sensitivity (>98%) with the various methods and is considered programmatically more efficient.
- It is standard practice to confirm a positive virological test result on a second specimen. However, for the purposes of clinical management a confirmed positive test from one specimen or a repeat test on the same specimen is viewed by experts as sufficient, provided that laboratory quality assurance is guaranteed.
- Testing before the age of 6 weeks using the DNA and RNA methods can reveal HIV in infants infected in utero but is not recommended for use in routine national programmes.
- The timing of any repeat testing should consider breastfeeding practices, as the risk of acquiring HIV infection from mothers continues throughout the breastfeeding period.

- Virological testing is also required for children presenting with signs and symptoms of HIV infection to health care services. Because of low PMTCT coverage, this is where most HIV cases present. It is recommended that, from 9 months of age, testing with HIV antibody tests be done first in order to ensure that virological testing is only performed on 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 when the purchase of diagnostic kits and equipment is being considered and when a virological laboratory is being chosen or upgraded to operate a diagnostic service.

Location of testing facilities

Deciding where and how many testing facilities are optimum depends partly on the national burden of HIV and the required coverage and capacity. The procurement of RNA or DNA (PCR or other) testing equipment for a small number of laboratories offers potential benefits, e.g. 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 a secure supply of consumables. However, the centralization of virological testing capacity may make the testing of specimens from remote regions slow and reduce the quality and reliability of the service. The optimum configuration depends on the factors outlined below and differs between countries.

Workload and sample throughput

The predicted workload and the frequency and number of specimens to be tested in a given period are crucial for assessing the cost-effectiveness and suitability of any given technology. Some assays are best for comparatively large throughputs and may lead to wastage if not fully utilized. Consideration should be given to both the immediate situation and the estimation of needs over the longer term (two to five years). Unless testing is performed regularly it is difficult to maintain the required level of competency among technicians, and problems associated with equipment are likely to occur more often that would otherwise be the case.

Increasingly, PCR methods are becoming automated, thus reducing the potential for errors and increasing the reliability of results. Moreover, real-time PCR is considerably shortening 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 begin with manual sample preparation and invest in full automation if their workloads increase. 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

Because viral nucleic acids may degrade over time the type of specimen and the time between specimen collection and testing are important factors, particularly if storage occurs at high ambient temperatures for extended periods, e.g. during transportation to laboratories. Specimens can be protected if transported rapidly at 2−10 ^{o}C. However, where transport and refrigeration are problematic, e.g. in remote or rural areas, the use of dried blood or plasma spots may be the best solution.

It is essential that the procedures be validated after implementation. For example, if dried blood or plasma spots are collected and transported to a diagnostic laboratory by mail, the promptness and reliability of the model should be evaluated at each point in the chain of events (collection, mailing, 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 a budget based on realistic objectives. A comparatively high degree of automation may be able to compensate for low staff capacity if the maintenance and repair of equipment can be assured.

Laboratory facilities and infrastructure

Various factors have to be considered when the selection or equipping of a laboratory to undertake HIV virological testing is carried out. Physically, the laboratory building should have reliable water and power supplies and appropriate storage facilities, including refrigerator 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. The responsibilities of 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 measures and waste-handling. Incineration is the preferred method for the disposal of PCR products. The 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 the quality of service at a high level, facilities should participate in both external and internal quality assessment schemes and should institute ongoing evaluation of laboratory performance. WHO and the United States Centers for Disease Control (CDC) can provide technical support.

Procurement, supply and maintenance

Various factors relating to the procurement of commercially available assays must be taken into account. For most resource-constrained settings the technology chosen should support the use of DBS samples with standardized and validated protocols. Some commercially available PCR platforms permit the measurement of hepatitis C and hepatitis B, other sexually transmitted infections, and *M. tuberculosis*. Multiple use of this kind may increase the cost-effectiveness of a given assay.

Because it is often possible to negotiate favourable prices through bulk procurement it may be advisable to conduct price negotiations at the national level. The WHO Bulk Procurement Scheme may be helpful in this regard. Suppliers are required to guarantee the timely provision of test kits and associated reagents and consumables with adequate shelf-lives. Negotiated packages should include technical support by the suppliers, 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 that is supplied.

Because of the cost and complexity of the technologies, laboratory managers should take a long-term view in their management of procurement and supplies, including forecasting needs, staggered deliveries ensuring sufficient stocks without wastage, and proper storage. The close monitoring of stocks makes it possible to accommodate increased demand and to avoid the interruption of supplies and services.

Further information

As prices and assay features are changing rapidly it is advisable for decision-makers and specialists to keep abreast of current information. The WHO Diagnostics and Laboratory Technology web site (http://www.who.int/diagnostics_laboratory/en/) is a useful resource, providing information on evaluated assays, bulk procurement, quality assurance, and guidance and training.

The WHO Department of HIV/AIDS web site includes a page on paediatric HIV infection (http://www.who.int/hiv/paediatric/en/index.html).

WHO contacts

WHO Geneva

Dr G Vercauteren
Essential Health Technologies
World Health Organization
Avenue Appia 20
CH-1211 Geneva 27
Switzerland

Tel: +41 22 791 47 28
Fax: +41 22 791 48 36
Email: vercautereng@who.int
Web: http://www.who.int/diagnostics_laboratory/en/

Dr Siobhan Crowley
Responsible Medical Officer
Paediatric and Family HIV Care
Department of HIV/AIDS
World Heath Organization
Avenue Appia 20
CH-1211 Geneva 27
Switzerland

Tel: +41 22 791 1609
Fax: +41 22 791 4834
Email: crowleys@who.int
http://www.who.int/hiv/paediatric/en/index.html

AFRO

Dr Gershy-Damet Guy-Michel
Regional Officer for Laboratory
Regional Programme on AIDS
WHO Regional Office for Africa
86 Enterprise Road
Highland
PO BOX BE 773
Harare
Zimbabwe

Tel: 263 23836096
Fax: 00 263 4 746127 or 791214 or 746867
Email: gershyg@afro.who.int

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